

THE  
BOTANICAL GAZETTE

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THE LIFE HISTORY OF *ZANARDINIA*

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 174

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(WITH TWENTY-FOUR FIGURES AND PLATES I-IV)

This paper deals with nuclear conditions in the life history of *Zanardinia collaris* Crouan, a monotypic genus which with the genus *Cutleria* constitutes the Cutleriaceae. *Zanardinia* is perennial and, like *Cutleria*, is characterized by large motile spores, whose formation is limited to a very short period of the year. On account of the shortness of this period and the consequent difficulty in getting reproductive stages, it was described as a species of *Zonaria* (AGARDH 1) belonging to the Dictyotales, but was placed in the Cutleriaceae under its present name when CROUAN discovered that it had gametangia identical with those of *Cutleria*.

Since AGARDH (1), several authors, including DERBÈS and SOLIER (7), CROUAN (4), JANCZEWSKI (11), and SAUVAGEAU (15), have described the outer morphology of the creeping and coriaceous thallus of *Zanardinia* and its multiplication by proliferation from the surface; and it must be admitted that some of these authors confused the young *Zanardinia* with immature stages of *Cutleria adspersa*, the two forms being confusingly alike in the juvenile condition.

REINKE (13, 14) was the first to describe the reproductive structures of *Zanardinia*. He observed, working at the Naples Station in 1875-1876, the actual fertilization of the female gametes by the male gametes. There seemed to be no apogamous germina-



tion of the unfertilized eggs. From the sporelings of both fertilized gametes and zoospores he obtained the filaments of *Zanardinia* which directly produce non-motile spores (*Secundärsporen*), a single one in each cell. In his cultures *Desmotrichum* appeared later, and consequently the ultimate fate of the sporelings and of the non-motile spores was not clearly determined.

SAUVAGEAU (15) is one of those who have done most work on the Cutleriaceae, having been publishing the results of cultures of *Cutleria* since 1898. He has shown that the thallus of *Zanardinia* is formed through the union of marginal filaments, and has described in detail the dorsiventral structure and the general topography of the constituent cells.

The first cytological paper was that of the writer, which was published as a preliminary note two years ago (21). The material was collected in the Bay of Naples in the winter of 1908 and the spring of 1909, during which time I occupied a table of the Carnegie Institution at the Stazione Zoologica. *Zanardinia* was found in the vicinity of Posilipo growing on the surface of rocks or sunken wooden blocks down to a depth of about 25 meters. Cultures of the plants and of their sporelings and fixation of critical stages were made in the laboratory of the Station. The study thus begun at Naples was finished at the Hull Botanical Laboratory of the University of Chicago.

The paper presents first the mitosis in the vegetative cells of the gamete-bearing plants, the formation of the gametes, the fertilization and germination of the fertilized female gametes, and the apogamous germination of unfertilized female gametes; then there is described the mitosis in the vegetative cells of the zoospore-bearing plants, the formation and germination of the zoospores; and finally, there is a brief statement concerning an alternation of generations in the life history of *Zanardinia*.

#### **Mitosis in the vegetative cells of the gamete-producing plants**

Gamete-producing plants of *Zanardinia* in early stages of development, while in the form of a concave disk or cup resembling *Peziza* and no larger than 1 cm. in diameter, showed numerous mitotic figures in the superficial layers of the thallus, no matter



what time of day or night the material was fixed. After the plant became 5 cm. or more in diameter, mitosis was much more frequent near the margin of the thallus or in hairs growing from the margin. After the plant reaches the adult size (8–10 cm. in diameter), the figures are very rare in the inner tissue and only occasionally found in superficial layers.

The cells of *Zanardinia* have quite thick walls, which are thickest in the huge inner cells and less thickened in the cells of the superficial layers that contain more numerous plastids. The plastids take stains with avidity, and it was more difficult to bring out the details of the mitotic figure than in *Cutleria* and *Fucus*. The nuclei in the resting stage, in almost any part of the thallus, are all about the same size and are a little larger than the plastids. Each contains a small, deeply staining nucleolus, which lies in the center. The remainder of the nuclear cavity is almost wholly occupied by a large body of karyolymph, and a few scattered chromatin granules lining the nuclear membrane.

One of the conspicuous features of the resting nucleus of *Zanardinia* is the fact that outside the membrane there are frequently a number of deeply staining globules of irregular size. These globules are so close to the membrane that for some time I was in doubt whether they were within or without the membrane. Careful study, however, showed clearly that certain of them are outside the membrane and yet in close contact

with it (fig. 1). As the nucleus increases in size and the chromatin granules within increase in quantity, delicate chromatin fibrils of irregular size appear among the granules. During this increase of chromatin granules, the deeply staining globules outside the membrane decrease, and finally disappear. A comparative study of many such cases has convinced me that the globules are quite closely allied to the chromatin and seem to pass readily through the nuclear membrane.

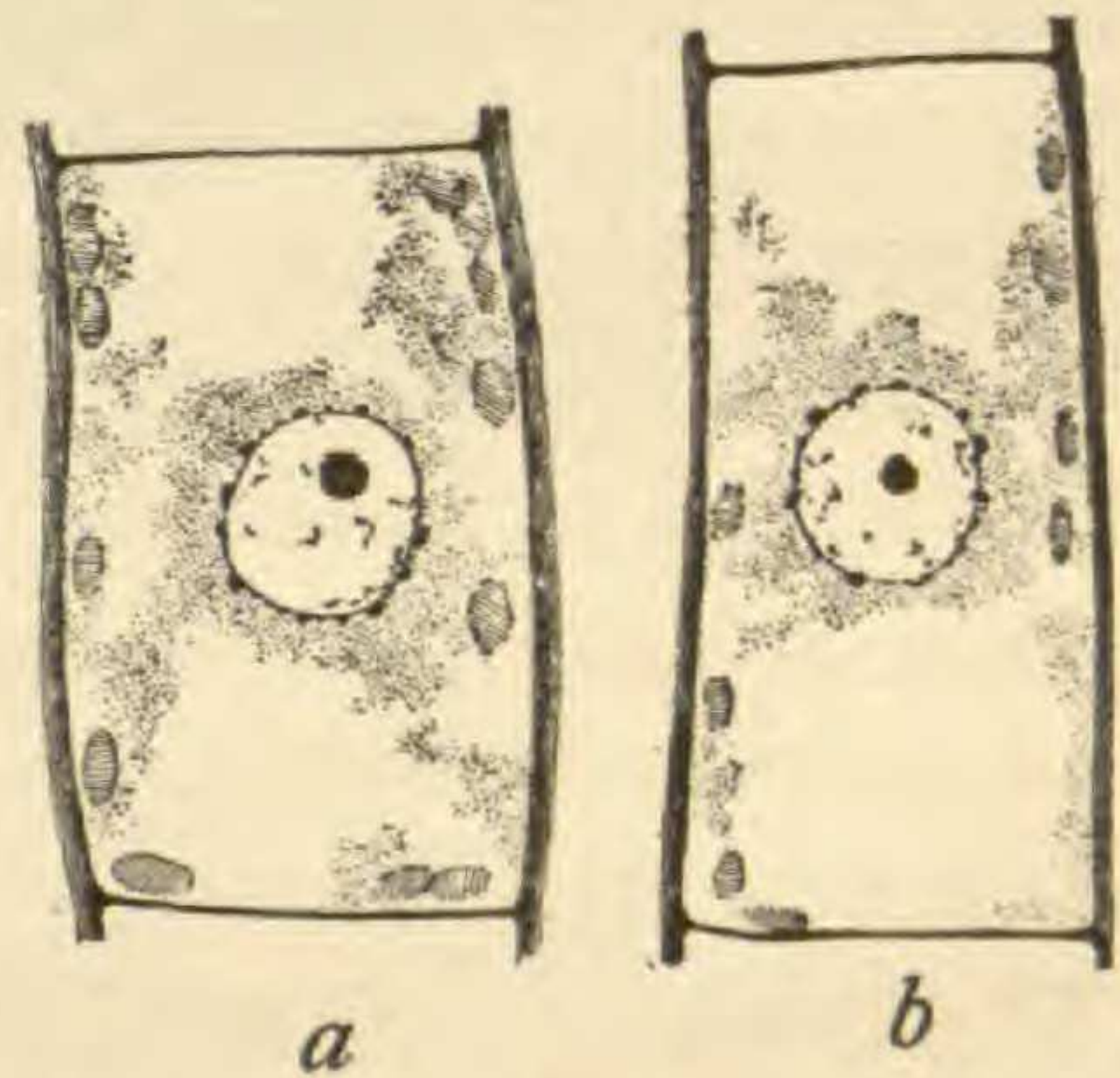


FIG. 1.—Portions of vegetative filaments: *a*, of zoospore-bearing plant; *b*, of gamete-bearing plant; nucleus with chromatic accumulations along the outside of the membrane.



Chromatin granules thus formed gradually move away from the membrane; the chromatin fibrils lying mixed with the granules form globules here and there; and the granules and globules then develop so that they become nearly spherical and uniform in size, being about 22 in number. They are chromosome primordia. The chromatin fibrils are all used up in the formation of chromosomes, and the nucleus contains only the chromosomes, a nucleolus, and the nuclear sap. When the chromosomes become arranged at the equatorial plate, kinoplasmic accumulations, developed from the cytoplasm surrounding the nuclear membrane, appear at the poles. A well marked centrosome-like structure in the kinoplasmic masses occurs only at metaphase, disappearing in anaphase. The chromosomes split longitudinally and half of each chromosome proceeds to each pole. During this process, the spindle is intranuclear. At telophase or at late anaphase, the nuclear membrane disappears and the two sets of crowded daughter chromosomes are surrounded by the cytoplasm, and the formation of the nuclear membranes follows. When the daughter nuclei are organized, the central spindle disappears completely. The cytoplasm lying between the two nuclei assumes a coarse, irregular alveolar structure, and the walls of the alveoli, probably after a change in their material, form a new cell plate. This process is similar to that in *Fucus* (20) and *Cutleria* (22).

#### Male and female gametangia

*Mature gametangia.*—Both male and female gametangia occur mixed on certain parts of the surface of the thallus (fig. 2). When the mature plant is living and fresh, the parts where the gametangia are borne are conspicuous from the deep dark color, as distinct from the deep brown of the general sterile surface. Both male and female gametangia are non-branched filamentous structures arising from superficial cells of the thallus. The male gametangia mature earlier than the female in the same individual. The upper surface of the plant, except the margin, is smooth and is devoid of any hairy growth. The patches of sori are composed exclusively of filaments ending in terminal gametangia. Occasionally, however, mixed with the ordinary gametangia are hairs which



in their middle region produce male or female gametangia, indicating that the latter are hairs in origin.

The mature male gametangium consists of a number of tiers of small cells (the male gamete mother cells), each tier comprising 8 cells, and since there are at most 33 tiers (fig. 4, *b*), the output of a single male gametangium is about 264 gametes. The mature male

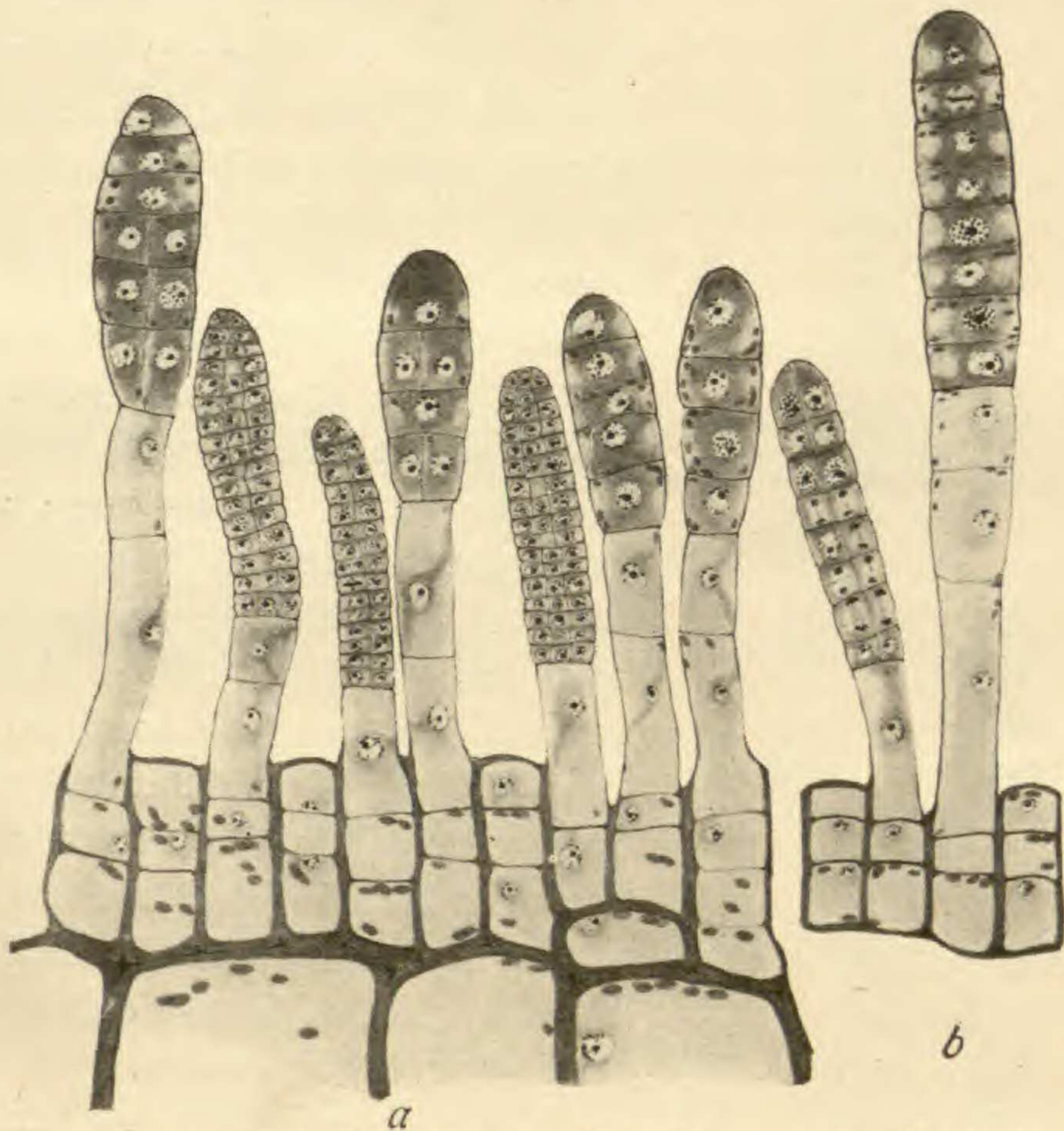


FIG. 2.—Portions of thallus with a number of filaments bearing both male and female gametangia: *a*, male and female gametangia with stalks composed of one or two cells; most of them are near maturity; *b*, young gametangia; a female gametangium with a stalk of three cells.

gamete in the free swimming condition outside the gametangium is oval and usually contains two plastids. A portion of one of the plastids lying laterally near the anterior end has a deep orange color, which is the red pigment, and in close association with this pigment are two cilia, one directed toward the anterior end, 6 times the length of the gamete, and the other in the opposite



direction, about 2.5 times the length of the gamete. The length of the entire male gamete is  $4.5 \mu$  (fig. 16, b).

*Development of the male gametangia.*—As stated above, male gametangia arise from superficial cells of the thallus. One of the

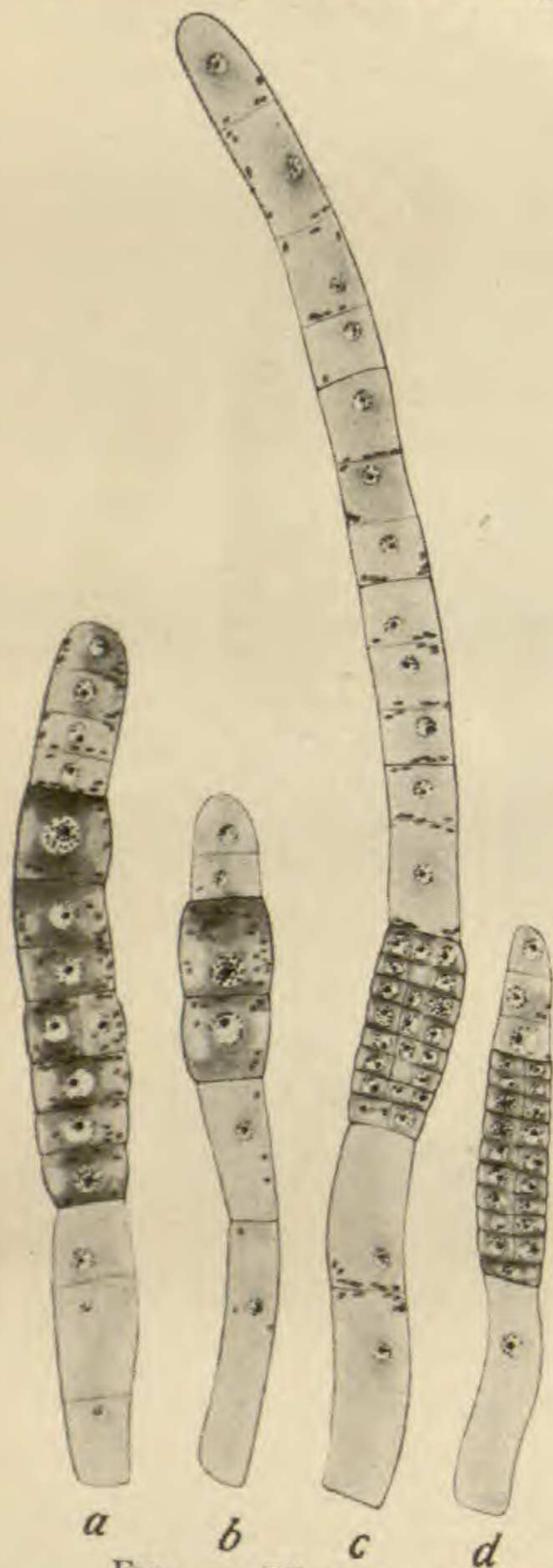


FIG. 3.—Filaments bearing gametangia in their middle region: a and b, with female gametangia; c and d, with male gametangia.

superficial cells divides, giving rise to a gametangium initial and a stalk cell. Often two or more subsequent divisions occur and there is produced a filament of two or more cells, the terminal one of which is a male gametangium initial (fig. 5, a, b, c). In rare cases a filament becomes a long multicellular hair consisting of a single row of cells, and some cell in the middle of the hair becomes the gametangium initial. This shows that the male gametangium is a hair in origin (fig. 3, c, d). This development of a male gametangium from a single superficial cell, occurring simultaneously in multitudes of neighboring cells on large areas of the surface of the thallus, results in the production of thousands of gametangia growing side by side, producing the dark-colored patches upon the thallus.

The nucleus of the male gametangium initial increases considerably in size. The chromatin network of the resting nucleus is marked by a number of knots mixed with fibrils. The chromatin knots increase gradually in size in prophase and finally break up into 22 chromosomes. When chromosomes become arranged at the equatorial plate, two distinct centrosome-like structures are present at the poles. The nuclear membrane either persists or disappears at metaphase (fig. 6, a, b).



The nucleus passes into telophase, two new daughter nuclei are formed, and then the subsequent divisions occur. The details were followed from the second division to the last, and the processes are simply a repetition of the first. When the young gametangium has reached the stage consisting of a single row of 7 or more cells, there occur also divisions perpendicular to the axis of the gametangia, so that each tier of the gametangium consists of 2 (fig. 6, *i*), 4 (fig. 6, *j*), or 8 cells (fig. 7, *a*). During these divisions the nuclei in the gametangium do not necessarily divide simultaneously, but often in quite irregular order (fig. 6, *c-g*). The size of the nuclei does not diminish in spite of the gradual and considerable diminution in size of the cell (fig. 6, *a-j*); for example, the figure in the first division in the gametangium (fig. 6, *a*) and that of the last division (fig. 6, *g, j*) are alike in size, while the first cell is ten times as large as the last.

During these mitoses in the gametangia, chromosomes were counted in prophase, in polar view of metaphase and anaphase, and the number is 22.

Regularity in the axes of these divisions, which take place either parallel or perpendicular to one another, results in producing the well known male gametangia of *Zanardinia*, comprising more than 30 tiers, each tier made up of 8 cells (fig. 7, *b*). Each individual cell in the gametangium is a male gamete mother cell, within which a single male gamete is formed. The mother cell contains a single large nucleus situated in the center, and usually two plastids whose position varies (fig. 7, *a, b*). The nucleus passes into a complete resting condition. One of the plastids moves near the nucleus and then a part of the plastid body becomes deep orange in color, which is the red pigment (fig. 7, *c*). When

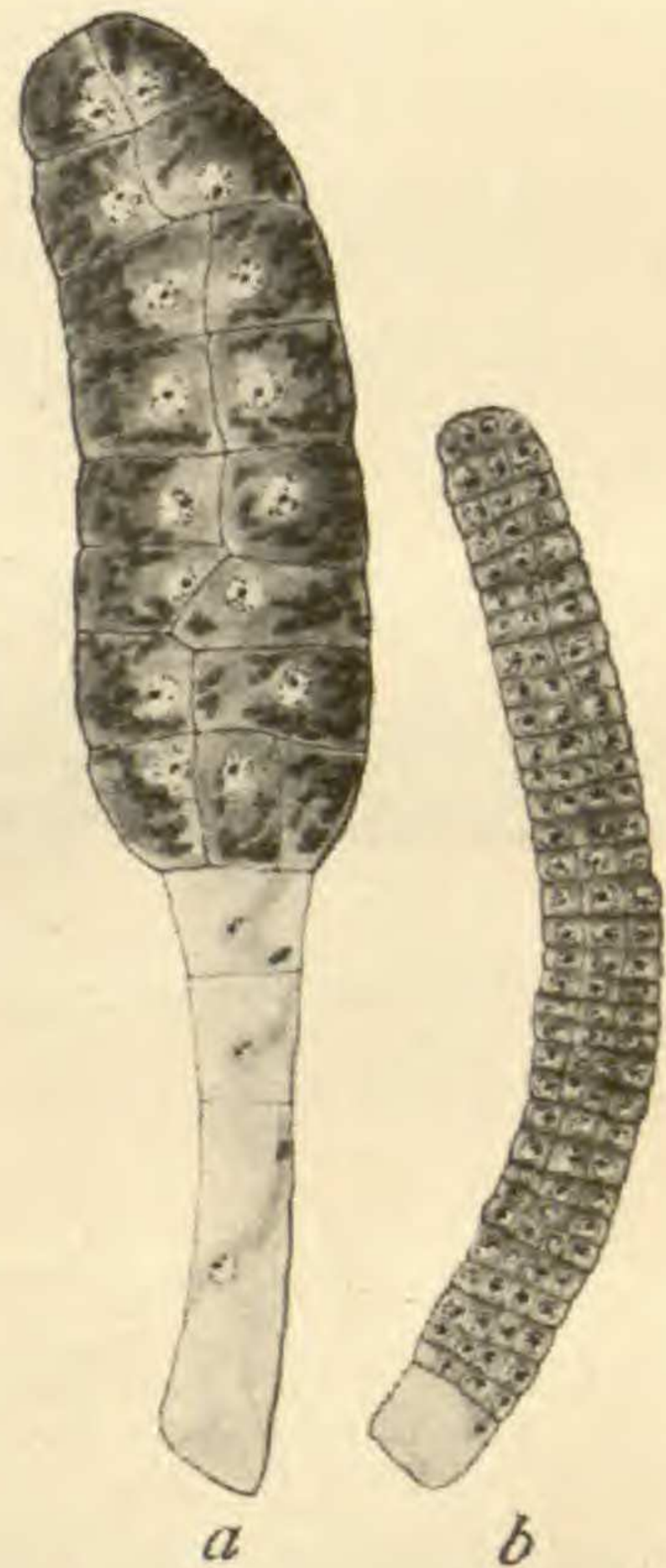


FIG. 4.—Mature gametangia: *a*, female gametangium with 8 tiers of gamete mother cells: *b*, male gametangium, with 33 tiers of gamete mother cells.



the male gamete is mature, a portion of the free surface of the membrane of the mother cell dissolves so as to leave a pore, through which the gamete is discharged (fig. 8). The cilia of the gamete first protrude from the pore, keep moving for a while, and then the whole body of the gamete emerges and is set free.

*Mature female gametangia.*—Mixed with male gametangia, there are developed female gametangia, which arise like the former from superficial cells of the thallus. The mature female gametangium consists of several tiers of mother cells, the number of tiers varying from 3 to 9 (figs. 2 and 4, *a*). Each tier comprises 2 or 4 mother cells, so that the output of gametes from a single female gametangium fluctuates between 6 and 36. The mature female gamete in

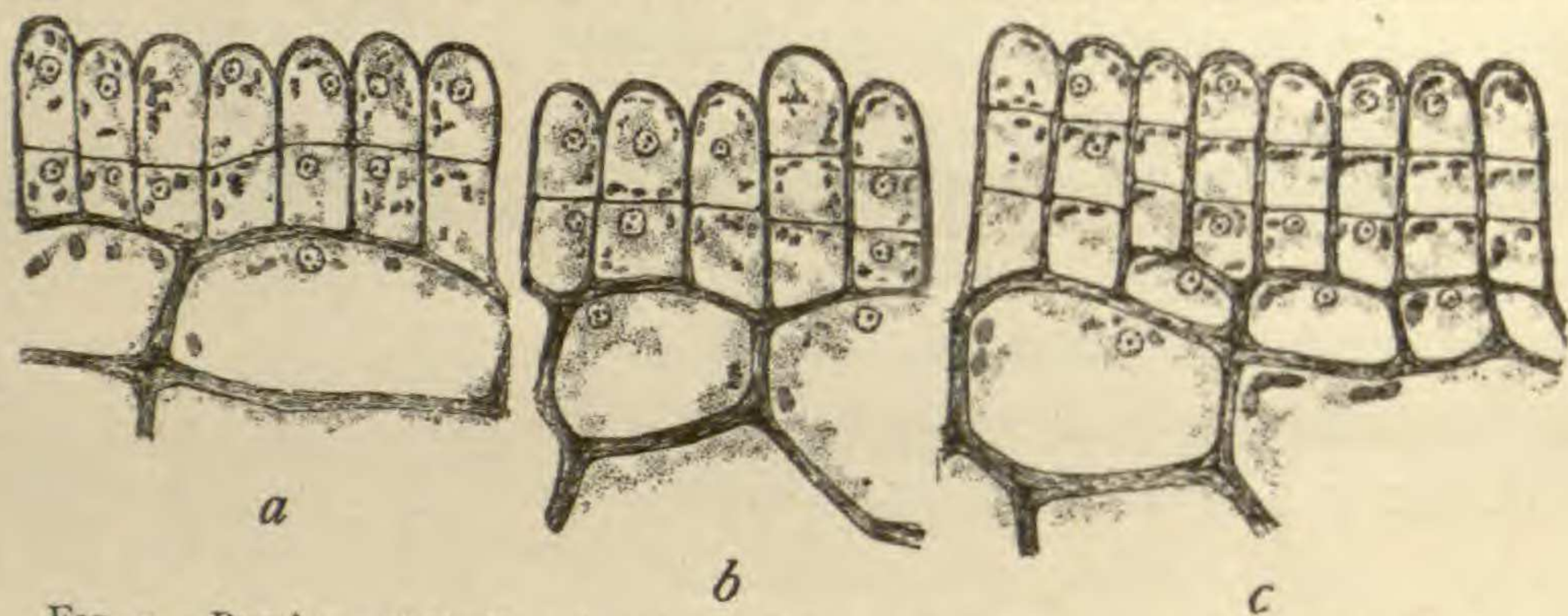


FIG. 5.—Portions of thallus showing origin of male gametangium initial: *a*, *b*, *c*, 2, 2 or 3, and 3 or 4 layers of superficial cells respectively, outermost of which in each case are male gametangia.

the free swimming condition outside the gametangium is oval (fig. 16, *a*) and usually contains less than 30 plastids. The anterior end of the body is destitute of plastids and consists of colorless granular cytoplasm, thus indicating polarity in the organization of the body. A portion of some plastid near the periphery in the anterior end takes up a deep orange coloring matter, which is the red pigment. Close to the pigment, two cilia are borne; one being directed toward the anterior end, 2.25 times the long diameter of the gamete, and the other in the opposite direction, 1.3 times the diameter of the gamete. The diameter of the female gamete is 19–23  $\mu$ . Active motility of the female gamete does not last long; at the longest under observation, the movement becomes sluggish within an hour, the shape becomes spherical (figs. 16, *c*, and 26), and the cilia are withdrawn or coalesce with the protoplast.



*Development of female gametangia.*—Like the male gametangium, the female gametangium arises from a superficial cell of the thallus. One of the superficial cells divides, giving rise to a gametangium initial and a stalk cell. A second or third division may be intercalated between the first division of the superficial cell and the

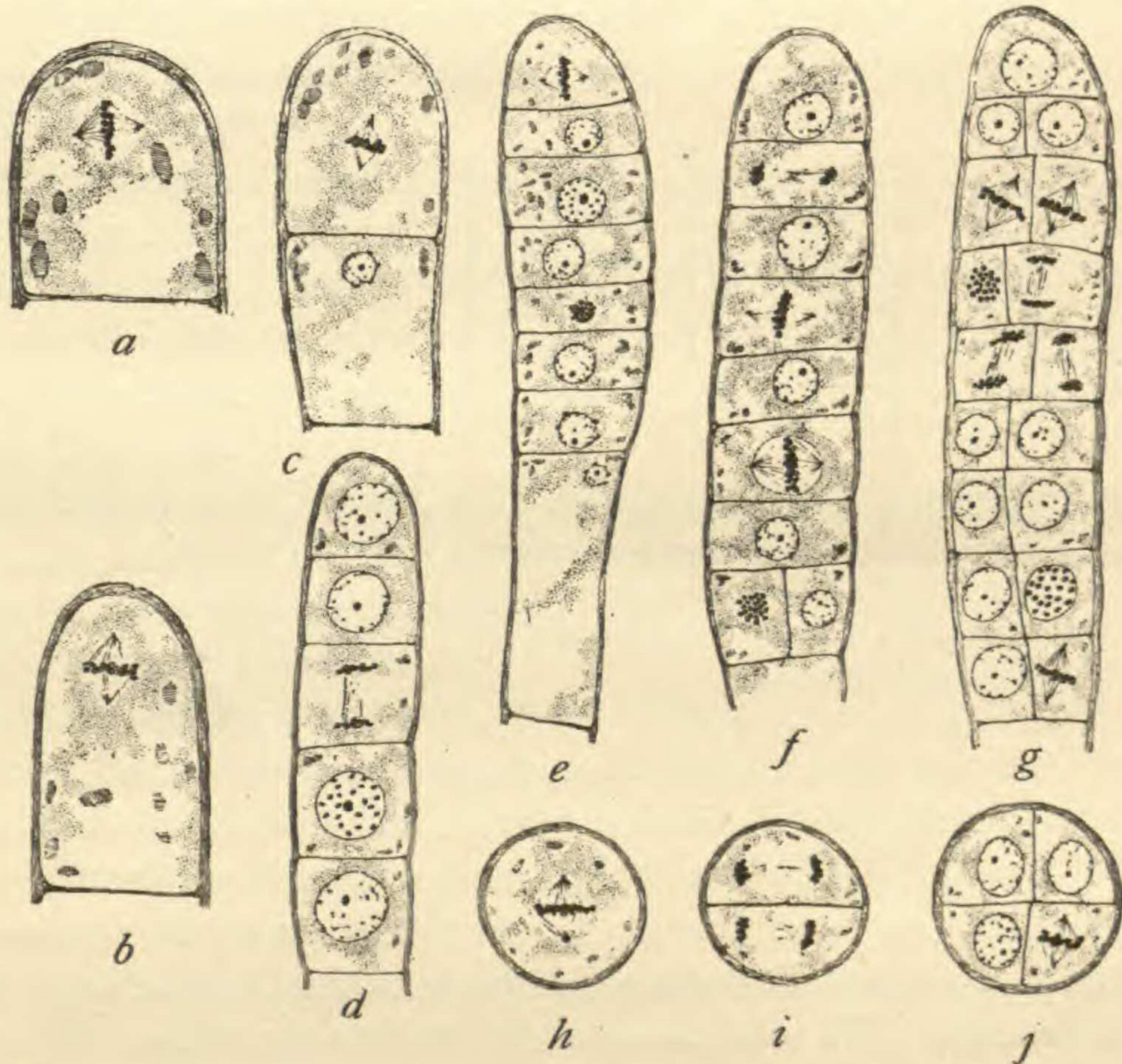


FIG. 6.—Male gametangia in various stages of development: mitotic figures in prophase, metaphase, and anaphase show 22 chromosomes: *a*, one of the superficial cells, whose division will result in two gametangium initials; *b*, first division in a gametangium initial; *c*, 2-celled stage; *d*, 5-celled stage; *e*, 7-celled stage; *f*, gametangium comprising 8 tiers; *g*, comprising 9 tiers; *h*, *i*, and *j* show respectively the cross section of gametangia of 1, 2, and 4 rows of cells.

differentiation of the gametangium initial, and in this case, the female gametangium has a stalk consisting of two or more cells (figs. 1 and 9, *a*, *b*). In rare cases, the female gametangium initial develops from a middle cell of a multicellular hair which has arisen from a superficial cell, indicating the hair origin of the female gametangia. The formation of the female gametangium takes place



simultaneously in multitudes of neighboring superficial cells, so that thousands of filamentous gametangia are produced, covering a considerable area of the surface of the thallus.

The nucleus of the female gametangium initial increases greatly in size, like that of the male, and becomes larger than the vegetative nucleus. The chromatin of the nuclear reticulum gradually increases and finally there is organized a prophase with 22 chromo-

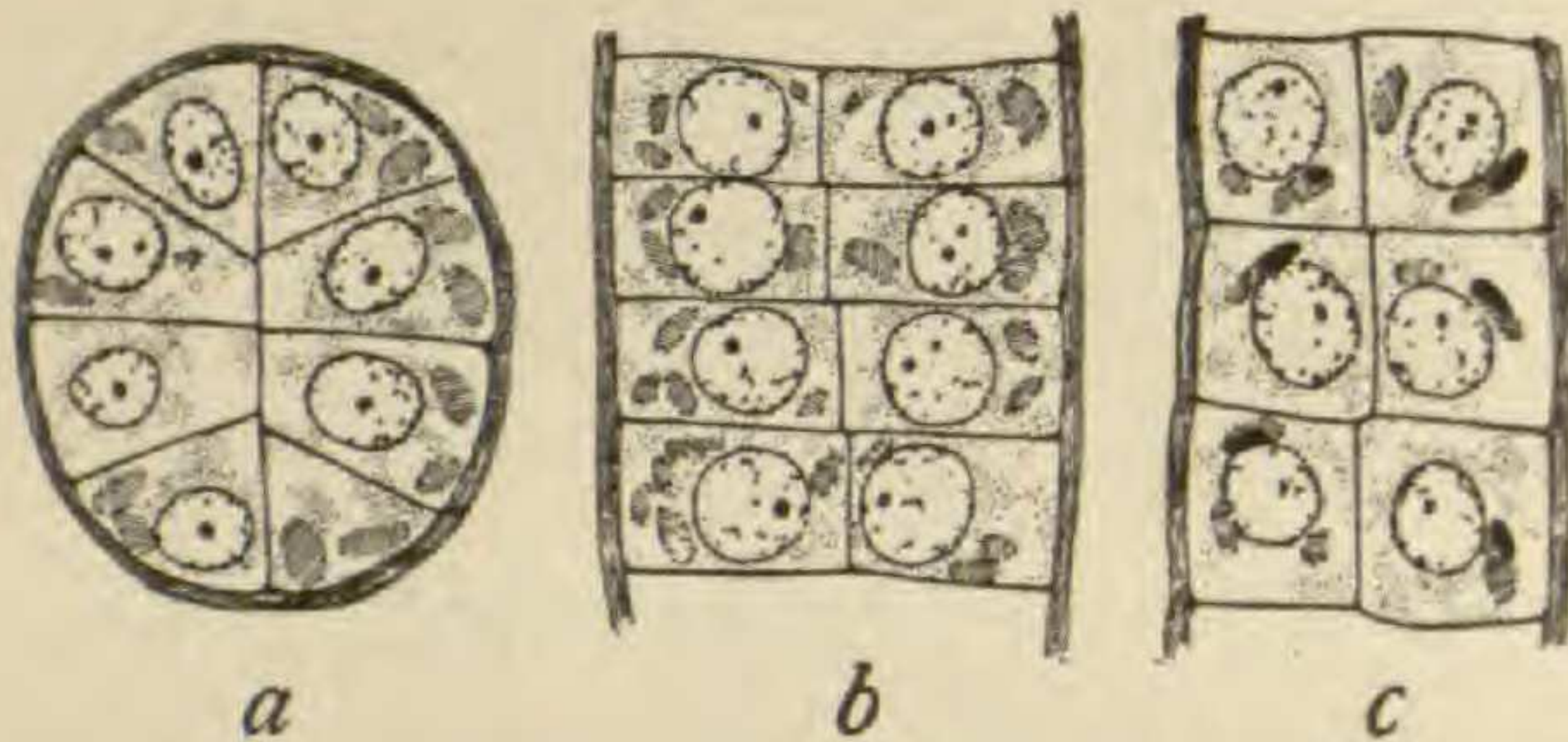


FIG. 7.—Mature male gametangia: *a*, cross section; *b*, tangential longitudinal section; *c*, optical section viewed laterally, in which red pigment spots have developed in a part of the plastids closely associated with each nucleus.

somes (fig. 10, *a*) and a single nucleolus. The nuclear membrane either persists or disappears at the time of the organization of the equatorial plate (fig. 10, *b*, *c*). The centrosome-like structures at the poles are present only during metaphase and early anaphase. At telophase, two new daughter nuclei are formed, and no central spindle remains between them. The cell plate is laid down by the cooperation of the vacuoles and by the transformation of cytoplasm.

The gametangium initial in the unicellular stage, whether male or female, shows no difference so far as the visible structure and size are concerned. The marked difference in the size of the male and female gametangia begins when the gametangia have reached the two-celled stage (figs. 6, *c*, and 11, *b*). The considerable growth of the cells in the gametangium is accompanied by that of the nuclei, and the details of the nuclear division are more readily and accurately followed during the gradual development of the female gametangium. As in the case of the male gametangium, subsequent divisions were followed up to the last division (figs. 11–15). The nuclear divisions in the cells do not take place simultaneously. When the gametangium has attained the three-celled stage, a cell in each tier divides transversely. One more transverse division may or may not occur; in the former case each tier consists of 4 cells (fig. 15, *b*), and in the latter it remains composed of 2 cells (fig. 15, *c*).



During these mitoses, chromatin globules accumulating outside of the membrane of the resting nucleus, and their gradual disappearance with the simultaneous increase of chromatin substances within the network, are beautifully shown. In prophase, in metaphase viewed from the pole, and in anaphase, 22 chromosomes are distinctly shown (figs. 11-14) proceeding toward the pole with equal rapidity.

The female gametangium comprising 3-9 tiers of cells, with 2 or 4 cells in each tier, is now established. Each individual cell of the gametangium is a mother cell and the whole contents of the cell become transformed into a single female gamete. The mother cell contains a large resting nucleus, surrounded by plastids. A portion of one of the plastids lying near the nucleus shows a deep orange color, which is the red pigment (fig. 14, *a, b, c*). When the female gametes are matured, a portion of the membrane of the mother cell dissolves, forming a pore through which the gamete is discharged. The cilia of the gamete first appear outside the pore, keep waving for a time, and then the gamete is set free.

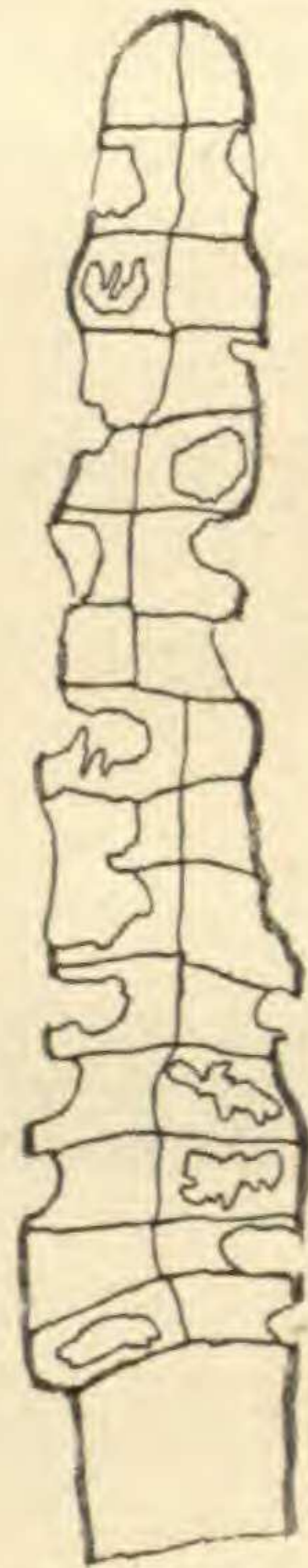


FIG. 8.—Mature male gametangium after escape of gametes.

### Fertilization and germination of the fertilized female gamete

The discharge of both male and female gametes occurs at almost any time during the day and night. Taking the case of a certain individual plant growing in a tank in the laboratory, the male gametangia matured and the discharge of the gametes began while the female gametangia were still in an immature condition, and did not mature and discharge their gametes until two or three days later. The time relations of the maturity of the male and female gametes in nature may be similar to that in artificial cultures. A periodic discharge of the gametes from the mature gametangia was observed to be most abundant at about 5:30 A.M., after which the discharge



gradually diminished, and finally ceased about 8:00 A.M. The male gametes continue in the motile condition for more than 24 hours, while the female gametes retain their motility for scarcely one hour and sometimes only for a few seconds. Toward the end of the motile condition, the movement of the gametes becomes sluggish, the cilia become coalescent with the protoplast, and finally the shape of the gametes becomes spherical. Even after the gametes have assumed the spherical form the polar organization in regard to the distribution of plastids is not lost. The area of granular cytoplasm with no plastids remains for a considerable time. From observation of the living material, it is evident that the formation of the wall, its

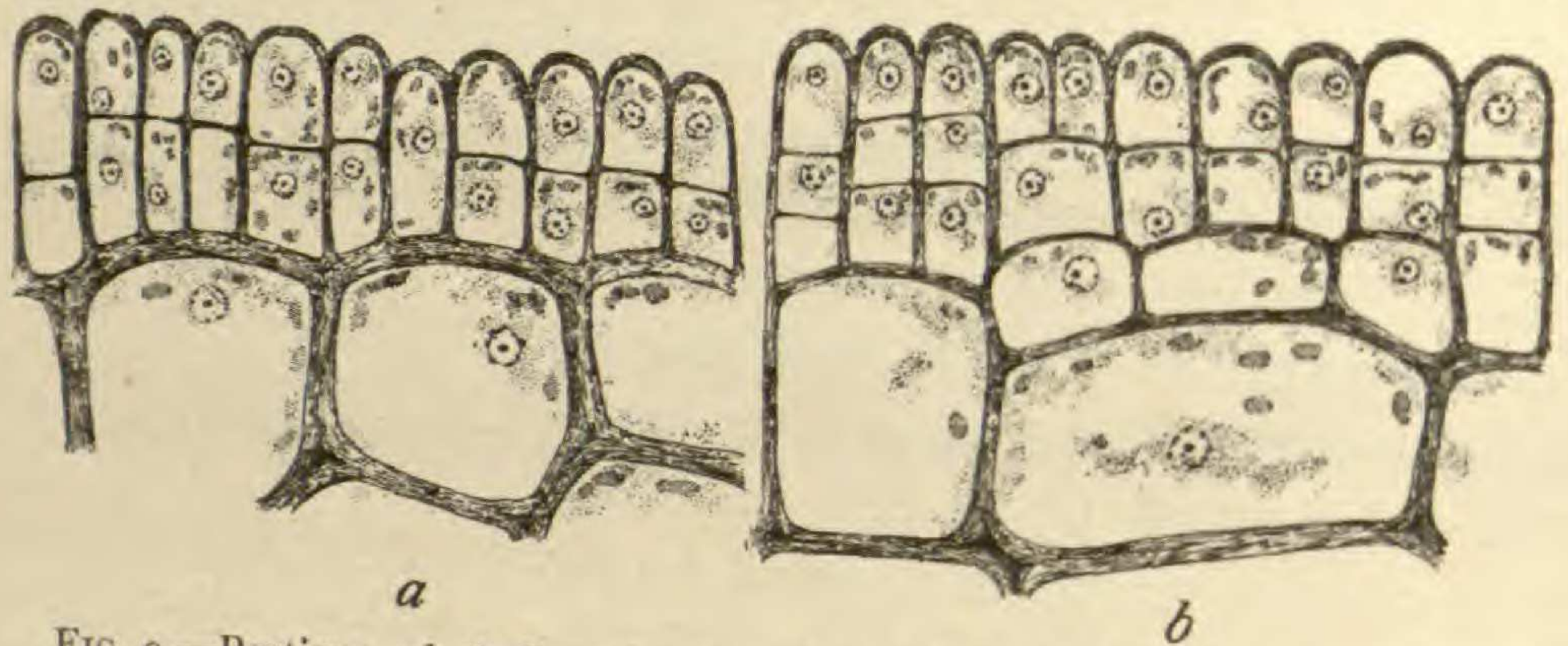


FIG. 9.—Portions of thallus showing the origin of the female gametangium initials: *a* and *b*, 2 and 3 layers of superficial cells respectively, outermost of which in each case are female gametangium initials.

subsequent thickening, and the elongation of the sporeling, both of fertilized and unfertilized female gametes, are in this area of the anterior end of the gamete. The union of male and female gametes and the succeeding nuclear behavior were studied in the material from artificial cultures as in my investigation of *Cutleria* (22). The fixations of the sporelings were made every 30 minutes for 24 hours, and then at 30, 36, 40, 46, 48 hours, and later every 5 days up to 30 days.

The male gametes while swimming freely become attached to the female gametes which are moving actively or sluggishly. Numerous male gametes, however, after swimming for a long period, even 24 hours, fail to come into contact with the female gametes, and then the movement ceases and the cilia fuse with the plasma



membrane of the body, which now becomes spherical. The nucleus, with a very delicate membrane, shows a number of chromatin knots identical with the number of chromosomes (fig. 25). It is a noticeable fact that the nucleus of the male gamete, during the period of active movement, is in the resting condition, and when the gamete becomes quiescent without any union with a female gamete, it still shows the same structure as if it had united with the female gamete.



FIG. 10.—Female gametangia: nuclei in the first division.

When the male gamete has just become attached to the female gamete, both gametes have very delicate plasma membranes.

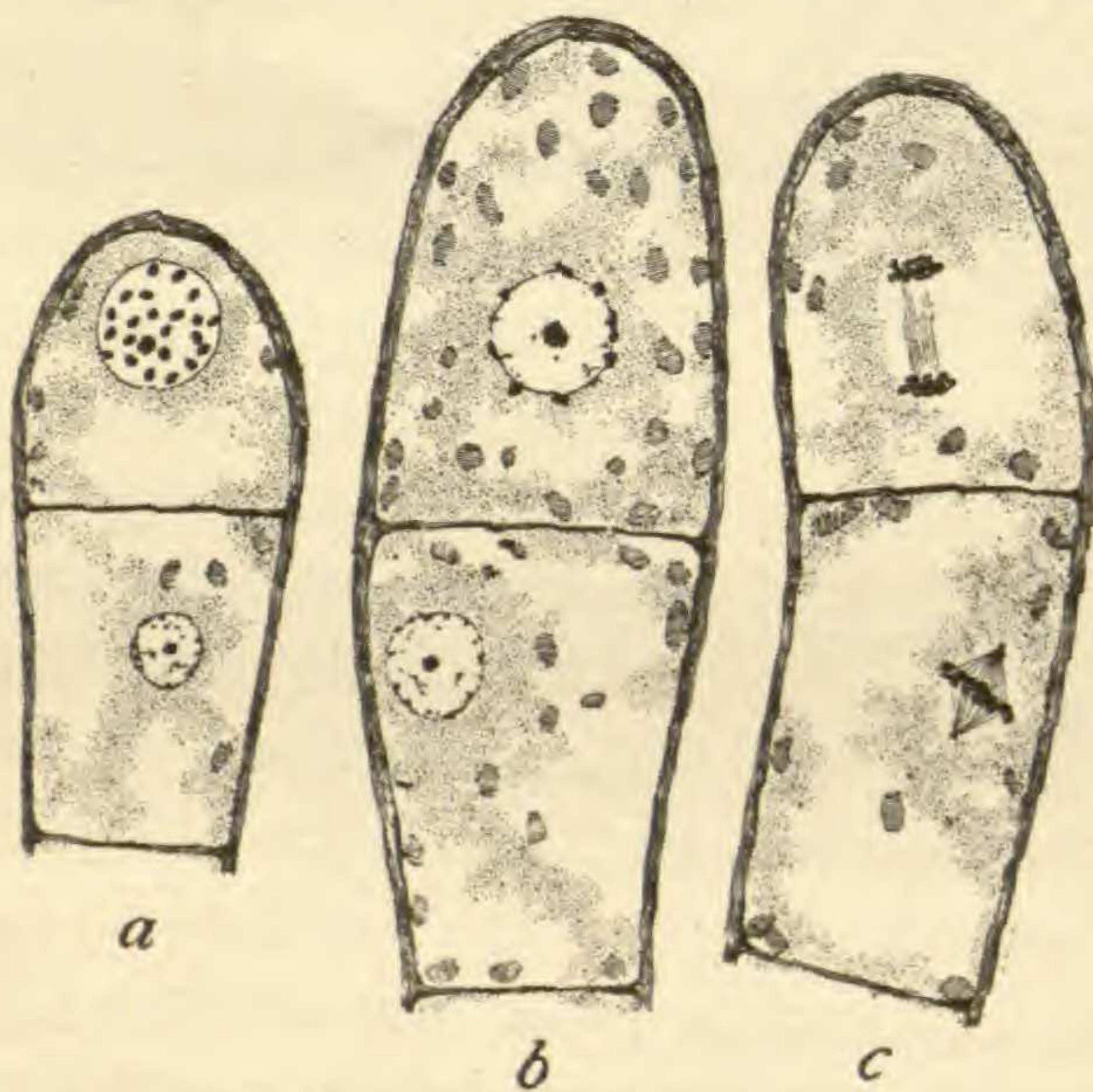


FIG. 11.—Female gametangia in 2-celled stage: *a*, nucleus in prophase, showing 22 chromosomes; *b*, nucleus with the accumulation of chromatin globules around the outside of the membrane; *c*, one nucleus in anaphase and the other in metaphase.

The nucleus of the female gamete is at the center of the cell, as in the resting condition, but that of the male gamete shows 22 independent chromosomes. The plasma membranes which lie between the cytoplasm of the male and female gametes become obscure and the cytoplasm of the two gametes comes into direct contact (fig. 27). The body of the male gamete can be observed for a short period as a protuberance from that of the female gamete (fig. 27).

Later, the protuberance is less prominent (fig. 28), and finally it is leveled down to the spherical curve of the body of the united gametes (fig. 29). The



male nucleus with 22 distinct chromosomes proceeds toward the female nucleus, which is in the resting condition (figs. 30, 31), until the male and female nuclei touch (fig. 32). The male nucleus is represented only by 22 crowded chromosomes closely applied to the periphery of the female nucleus (fig. 33); each chromosome of the male nucleus enters into the female nucleus (fig. 34); and finally each chromosome becomes vacuolized and occupies a part of the female nucleus (fig. 34). Later, the fusion nucleus shows no place-distinction of network of both male and female origin.

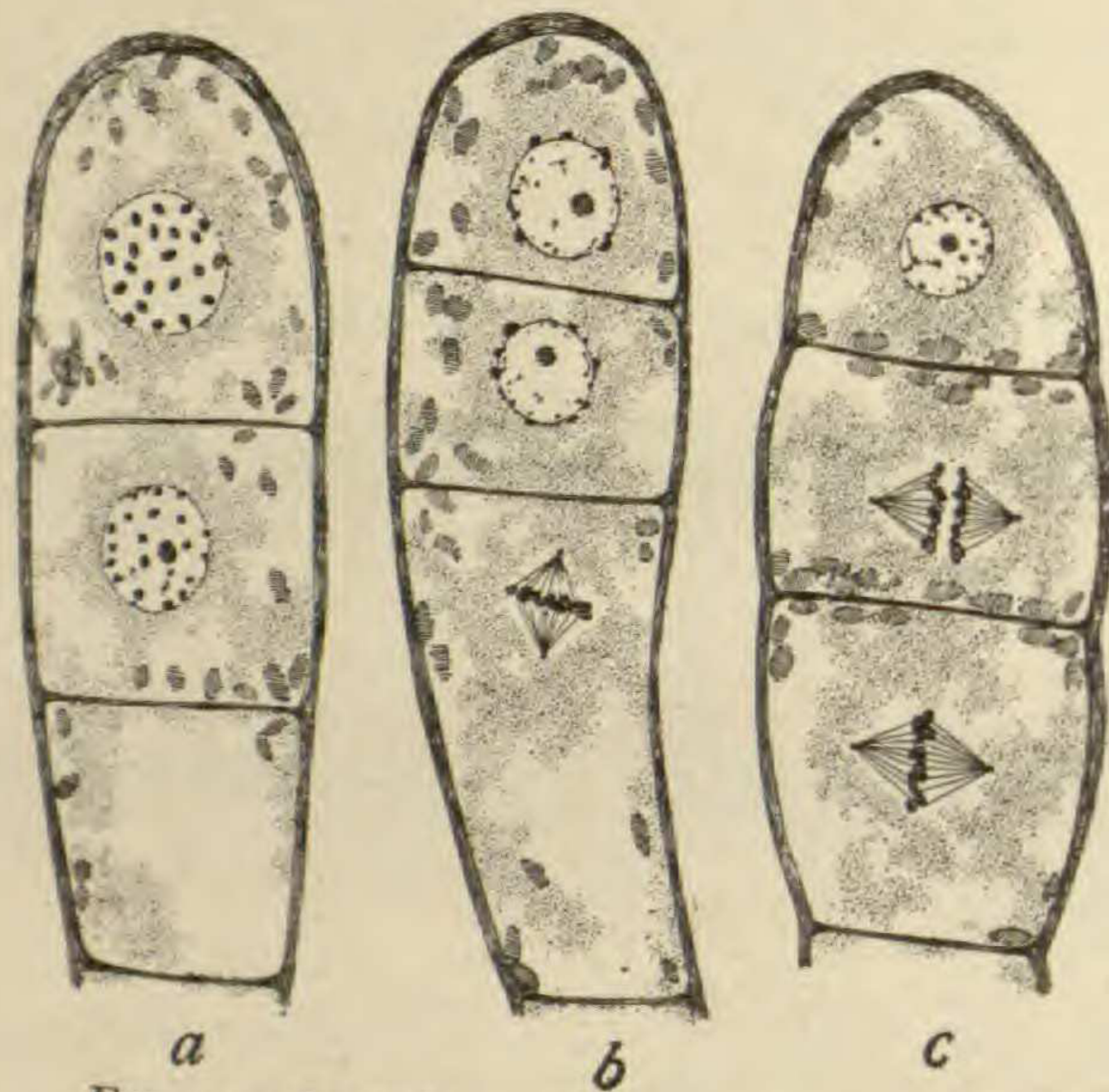


FIG. 12.—Female gametangia in 3-celled stage: *a*, 2 nuclei in prophase, showing 22 chromosomes; *b*, 2 nuclei with chromatin globules around the outside of the membrane; *c*, 2 nuclei in metaphase.

forming a structure in which the male and female constituents cannot be differentiated by staining.

The formation of the cell wall around the protoplast of the zygote is gradual. Neither the union of the male and female cytoplasm, nor the union of the male and female nuclei seems to be necessary to the formation of the wall, because there is great variability in the interval between the formation of the wall and the process of protoplasmic union, and, moreover, in the cases of zoospores and unfertilized female gametes, the wall is perfectly formed.

The first segmentation division of the sporeling from the fertilized gametes takes place about 24 hours after the union of the

It is a question whether the reticula of male and female origin do occupy distinct places, lying side by side in the fusion nucleus, or whether they intermingle and resume their individuality at the time of chromosome formation. In any event, the fusion nucleus passes into a complete resting condition, with chromatin knots of various sizes and shapes, together with delicate, irregular, discontinuous fibrils,



gametes. In early prophase, the nucleus shows 44 chromosomes, all alike both in size and in shape (fig. 35). In middle prophase the chromosomes become more compact (figs. 36, 37). During the formation of the spindle, the nuclear membrane disappears and the equatorial plate is established (figs. 38, 39). The polar view of the plate shows 44 chromosomes (figs. 40, 41). Each chromosome splits longitudinally and half of each proceeds to each pole (figs. 42, 43). The growth of the sporelings holds no strict relation to the mitosis within; the mitosis may take place before the sporeling begins to elongate (figs. 17, 39, 43), or more often the sporeling elongates while the nucleus is in the resting condition (figs. 17, 34). The axis of the first division, as a rule, is perpendicular to the growing axis of the sporeling. After telophase the sporeling is divided into two cells (fig. 44). The nuclei in the two cells divide either simultaneously (fig. 49) or one after the other (figs. 45-48). The number of chromosomes appearing at

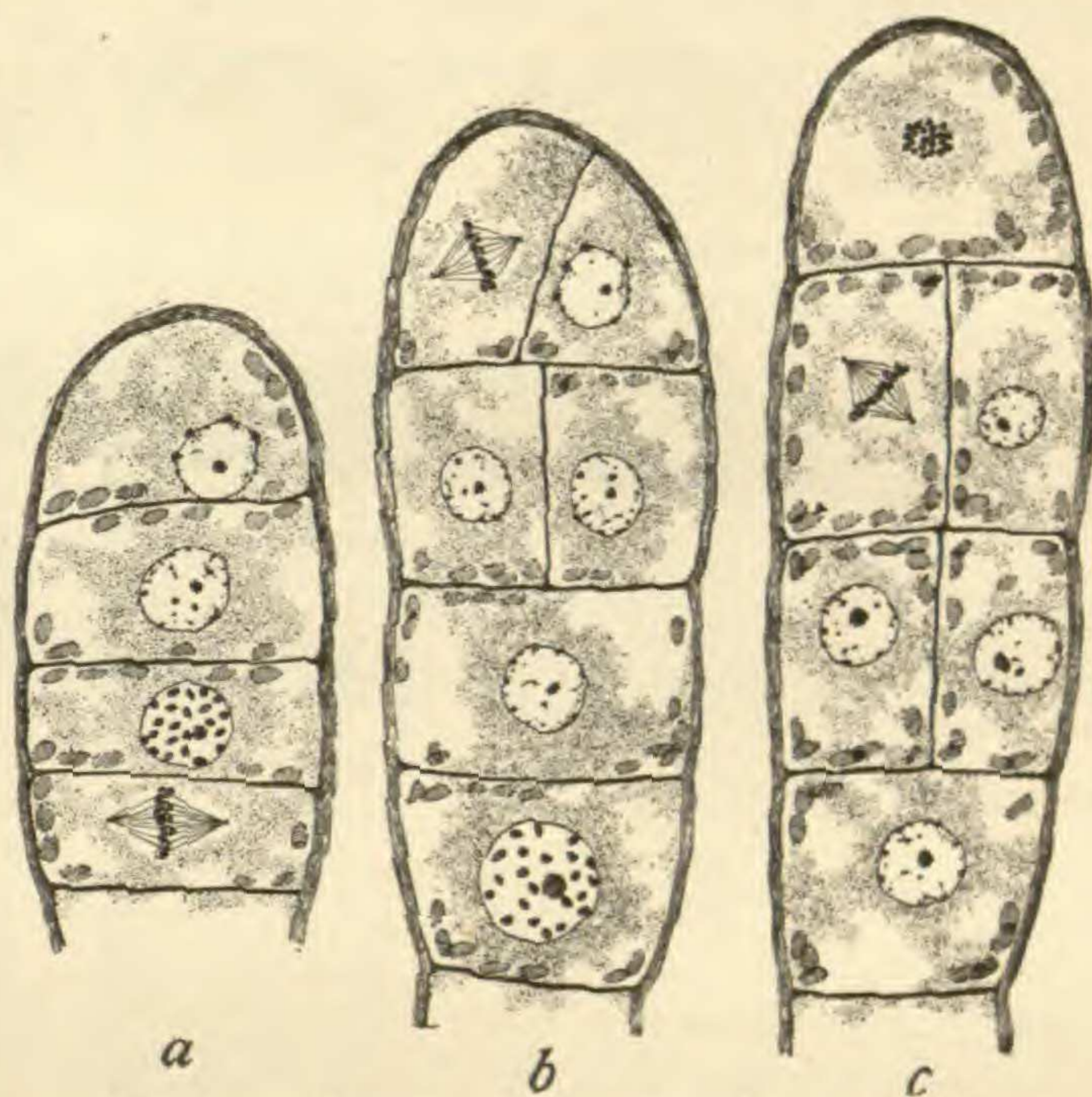


FIG. 13.—Female gametangia with 4 tiers of cells: *a* and *b*, a nucleus in each with chromatin accumulations around the outside of the membrane and a nucleus in each in prophase, showing 22 chromosomes; *c*, nucleus in anaphase viewed from the pole, showing 22 chromosomes.

prophase (fig. 44) and metaphase (fig. 45) is 44. After the 3-celled stage, the growth of the sporeling is not very uniform. The numerous cases observed showed great variability in the method of developing into the multicellular condition. But one principal fact that holds true in almost all cases is that when the sporeling has reached the 2 or 3-celled stage, the cell divisions occur chiefly in a single terminal cell or in two upper cells, while the basal cell



never divides further. The basal cell is an elongated portion of the sporeling, predetermined in its unicellular stage. Some of the sporelings up to the 7-celled stage are shown in fig. 17.

The sporelings in the cultures, after the 10 or more-celled stage, continue to develop in one direction. The cultures were watched and examined carefully every 5 days up to 30 days, when the plant was still in the filamentous condition (fig. 18, *a*). The filament with a holdfast at the base is unicellular, except near the base,

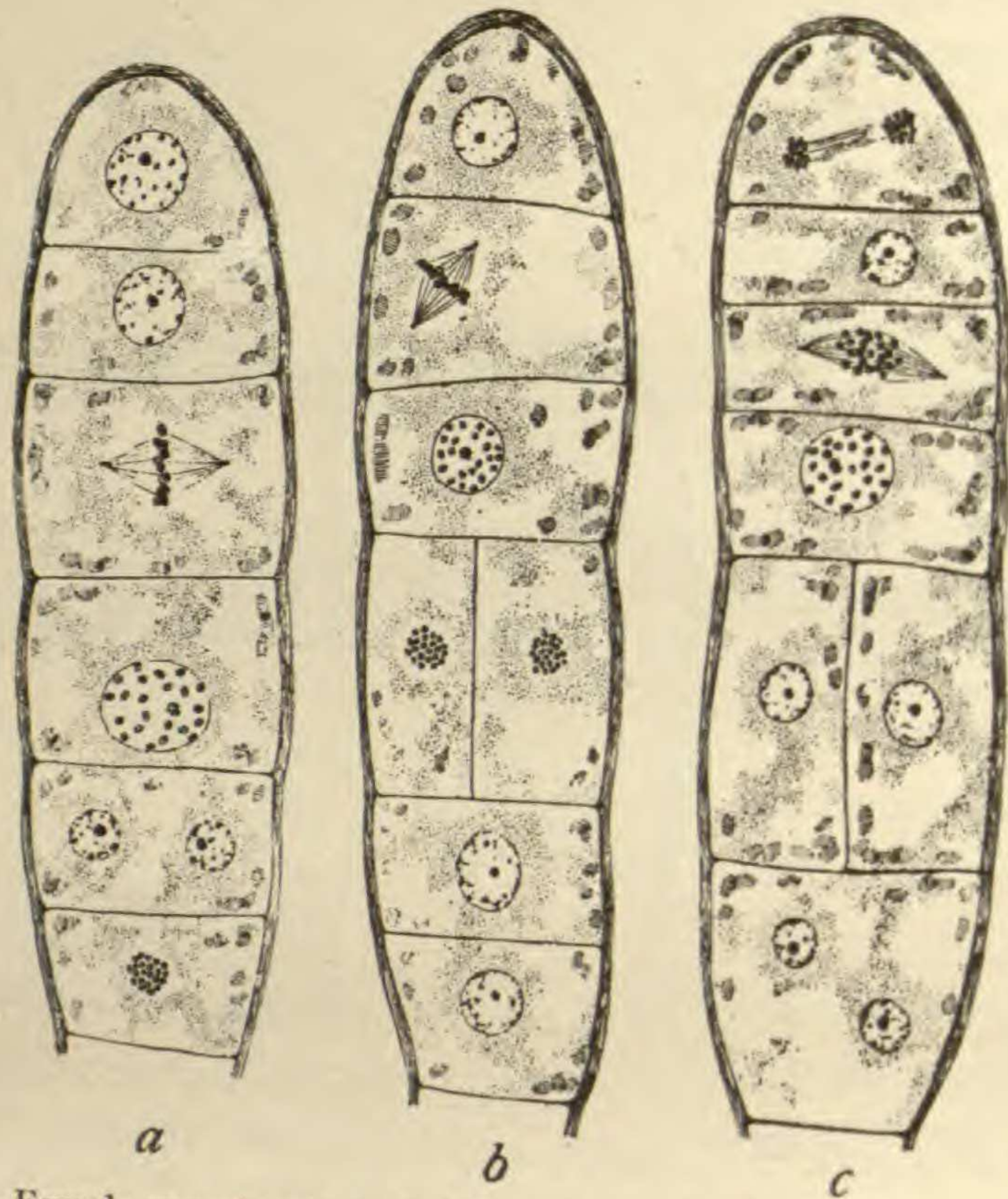


FIG. 14.—Female gametangia in 6 tiers of cells: *a*, *b*, *c*, a nucleus in each in prophase and some in anaphase viewed from the pole, showing 22 chromosomes.

where it is often multicellular. The filament is strikingly like the early stage in the sporelings of *Aglaozonia*. Later, this primary filament does not continue in the upward direction, but produces laterally at its base a number of filaments one after another and side by side, which fuse so as to form a funnel or cup, expanded upward and narrowly constricted downward. Upon the expanded upper margin of this shallow cup, the terminal parts of these filaments remain as hairs. The structure thus produced in the cultures



presents a striking likeness to the young plant of *Zanardinia* in nature, as it occurs thickly on the rock or broken wooden blocks in sea water.

### Germination of the unfertilized female gamete

As previously stated, the female gametes after their discharge from the gametangia may come to rest very shortly or swim for as long a time as one hour. At the end of the movement, the female gamete becomes spherical. If the female gametes have failed to be caught by the motile male gametes, they remain as motionless,

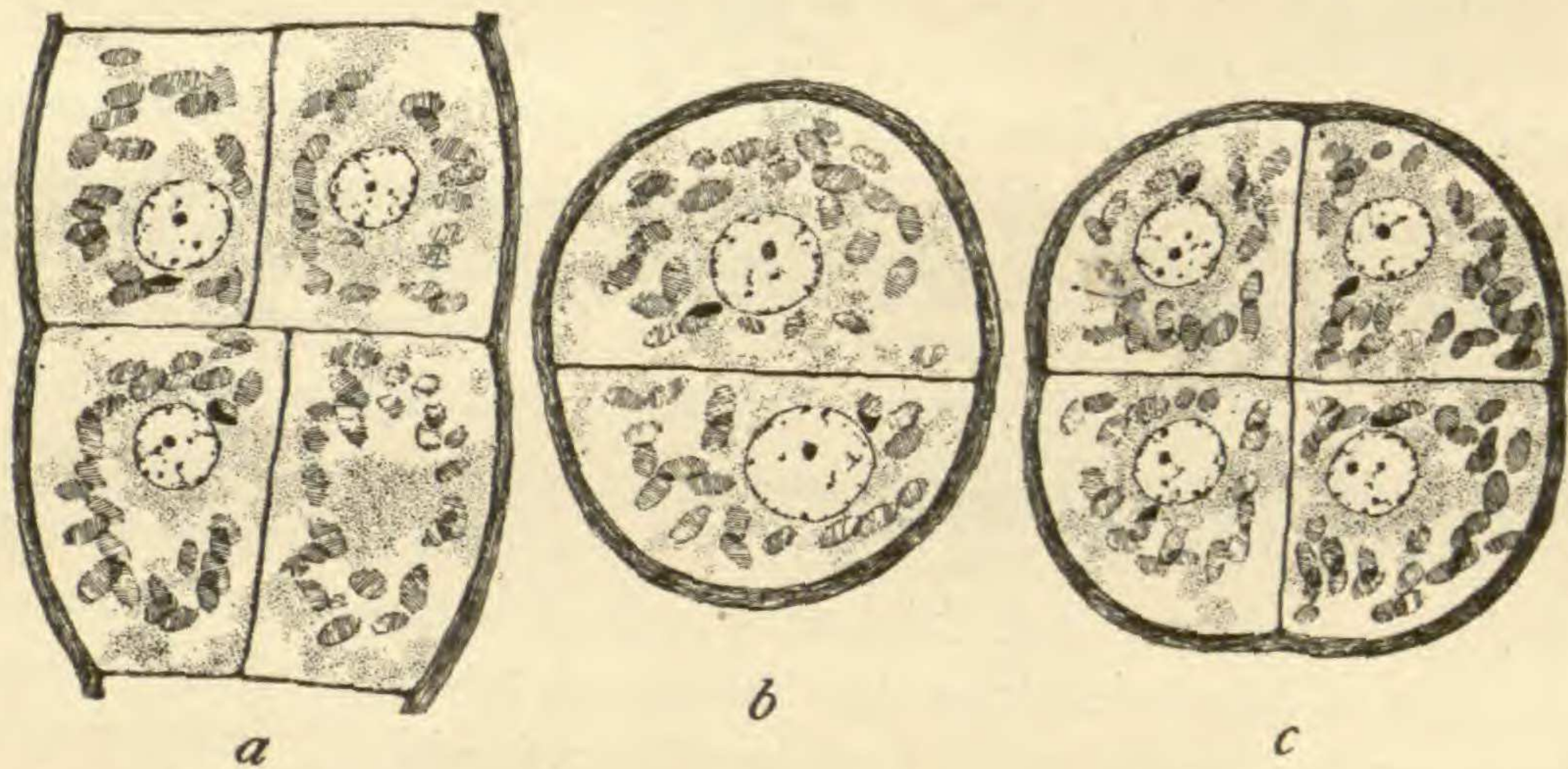


FIG. 15.—Portions of mature female gametangia: *a*, longitudinal section; *b* and *c*, cross sections.

spherical bodies for a considerable time. If they have been fertilized, their nuclei may divide within 24 hours, but when fertilization has not occurred, mitosis is delayed. Even 30 hours after the quiescence, no wall has been recognized (fig. 50). About 46 hours after quiescence a thin wall is developed, and when about 48 hours old, nuclear division begins (figs. 52-57). Every phase in this division is typical and the number of chromosomes is clearly 22 (figs. 52, 55). The elongation of the sporeling begins about 46 hours after quiescence. At the 2-celled stage, one of the two cells which has a thickened wall at its free surface divides once or not any farther, and the cell corresponds to the elongated portion of the sporeling at the unicellular stage. The other cell, which has



no particular thickening of the wall, divides successively and gives rise to all of the subsequent structures. The general morphology of the sporeling in its subsequent development (fig 19) is like that of the fertilized female gamete. The late development of the sporeling was followed up to the stage consisting of about 100 cells. The sporeling at this stage is a filamentous structure whose outer morphological character is hard to distinguish from the filamentous product of the fertilized gametes. Whether the products of the apogamous sporelings would reach maturity is not yet determined.

#### **Mitosis in the vegetative cells of zoospore-producing plants**

Zoospore-producing plants of *Zanardinia*, in their external morphology, do not differ from gamete-producing plants except in their reproductive organs. The general morphology of the cells composing the thallus is alike in zoospore-producing and gamete-producing plants. The size of the cells in the superficial layers, where the reproductive organs originate, was measured in both plants and was found to be the same.

Vegetative mitosis was studied in the cells of young plants 1.5 cm. in diameter, and in older plants 7 or 8 cm. in diameter. The cells of the hairs upon the margin of the adult plants were also favorable for the study of vegetative mitosis.

The size of the resting nucleus in the superficial cells is about the same as that of the plastids or is even smaller. That the chromatin network is represented chiefly by a number of irregular knots, that the deeply staining globules attached to the outside of the membrane gradually diminish as the quantity of the chromatin knots within increases, that centrosome-like structures are conspicuous only at metaphase and early anaphase, and that the formation of the cell plate at telophase is by means of alveoli of the cytoplasm, are features which repeat almost exactly those described for the gamete-producing plants. The fundamental difference, however, was the appearance of 44 chromosomes, which were counted accurately in prophase and in polar views at metaphase and anaphase.

On account of the cell organization, the zoospore-forming individual of *Zanardinia* cannot be considered as the homologue



of the gamete-producing form, although their similarity might mislead those who observe only the external features.

### Formation of zoosporangia

Zoosporangia are produced on the upper surface of the thallus. In the mature plants, the groups of zoosporangia are distinguished by patches of darker color which contrast sharply with the light-brown color of the sterile portion. These patches are composed of thousands of zoosporangia, produced side by side upon the thallus. The patches look darker than the rest because the sporangia, no matter what their age, contain a great number of plastids which have an olive-brown color. The production of zoosporangia begins at a certain spot and proceeds centrifugally, so that the younger

stages are generally found at the edge of the patches. The details of the origin of the zoosporangium are as follows: A superficial cell of the thallus begins to swell, elongates slightly and divides, giving rise to two cells, the upper one a zoosporangium initial or zoospore mother cell and the lower one a stalk cell (fig. 20, *a*). The process occurs simultaneously or successively in a number of neighboring superficial cells, so that finally zoospore mother cells are produced in great numbers, crowded closely together. When the superficial



FIG. 16.—Gametes sketched from living material: *a*, female gamete; *b*, male gamete; *c*, female gamete which has assumed the spherical form; *d*, fertilized female gamete; *e* and *f*, 2-celled sporelings from fertilized gamete; in the sporelings large pigment spots are derived from the female gamete and small ones from the male gamete.



cell divides 1-3 times more, thus producing 3-5 layers of superficial cells, the uppermost cell becomes the zoospore mother cell (fig. 20, *b, c*). Frequently several cell divisions take place in the superficial cell, so that a superficial cell develops into a filament consisting of more than 7 cells, the terminal one of which becomes the mother cell (fig. 21, *a*). Moreover, one of the superficial cells, unlike its neighboring cells, may develop into a long multicellular

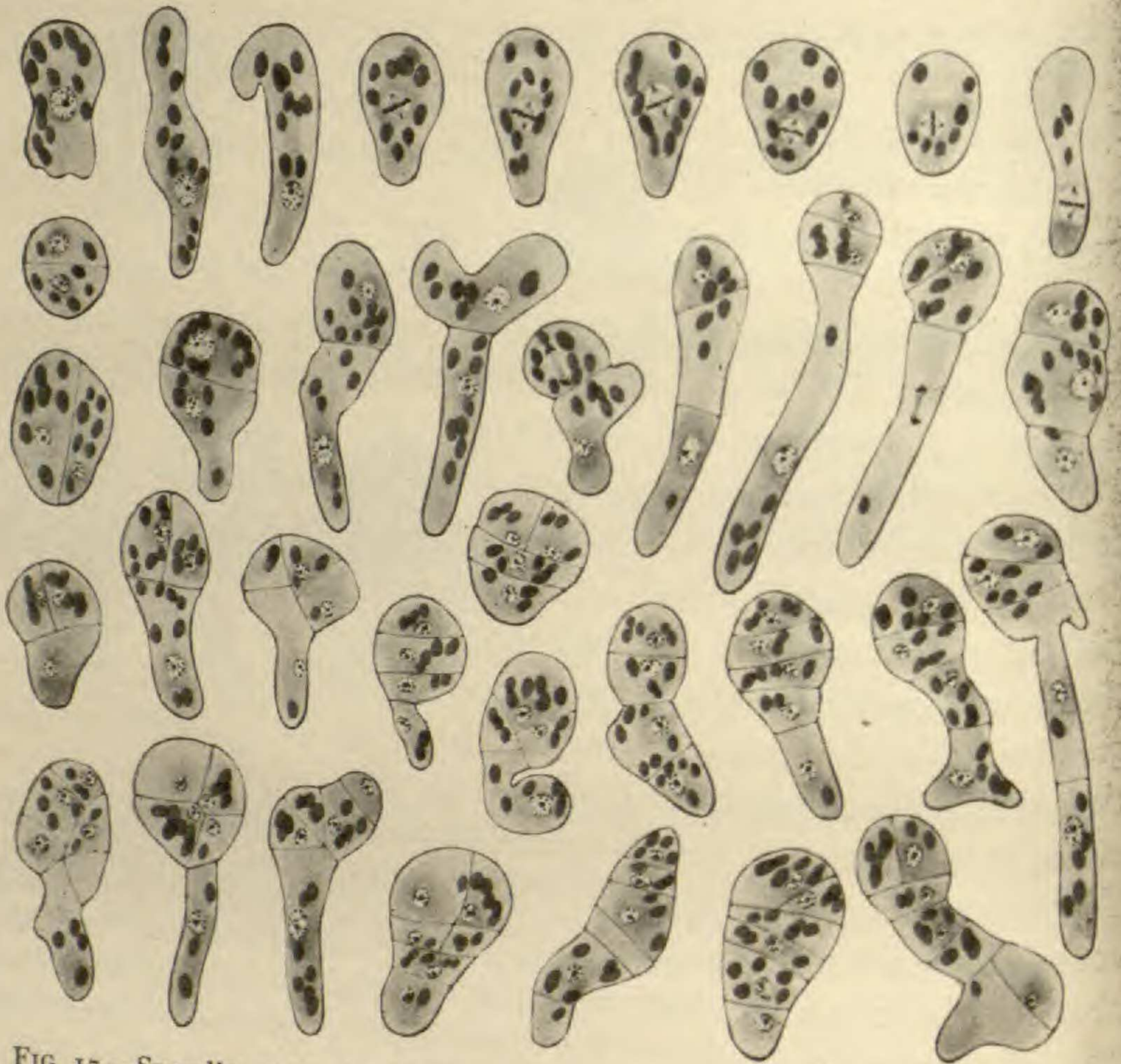


FIG. 17.—Sporelings from fertilized gametes: development in length is dominating.

sterile filament (fig. 21, *b*), thus indicating the hair origin of the zoosporangia of *Zanardinia*.

The nuclear division of the superficial cell which gives rise to the mother cell and stalk cell is typical, and 44 chromosomes are present (fig. 75). The zoospore mother cell or zoosporangium from a very early stage is distinguished from the sterile superficial cells by being longer than wide (fig. 76). The length of the mother



cell increases as it grows, until it reaches three times its width; then the nuclear changes begin. By this time the mother cell assumes its characteristic ellipsoidal shape, slightly swollen at the top. The nucleus grows with the growth of the cell (fig. 77).

The nucleus in the resting condition contains delicate chromatin fibrils and a nucleolus. Outside the membrane and tightly applied to it there are deeply staining globules (fig. 77). As the chromatin fibrils inside the nucleus grow in quantity, the number and amount of the deeply staining globules diminish (fig. 78). Finally there appear chromatin threads stronger and more continuous than before, and the globules lying outside the membrane disappear completely. It is possible that these globules consist of material closely allied to chromatin, and that they pass into the nucleus, thus contributing to the formation of chromosomes (fig. 79).

The chromatin threads, which are continuous for a considerable distance, run irregularly through the cavity and become more uniform in thickness (fig. 80). They gradually become arranged near the membrane, their parts running parallel by repeated bending (fig. 81), and finally there are established a number of loops of different sizes centering at one part of the membrane (fig. 82). These loops shorten and thicken (figs. 83-85). The loops now show double arms lying side by side; each arm of the loop is a single structure, its origin having been traced from the first indication of a thread structure direct from the chromatin fibrils. The transverse section of these parallel grouped loops is shown in fig. 86; the cut ends of two arms of one loop lie closer than the cut ends of the arms of another loop, and the number of the ends is about 88. The shortening and thickening of the loops proceed, and then they are gradually detached from the main group and form paired chro-

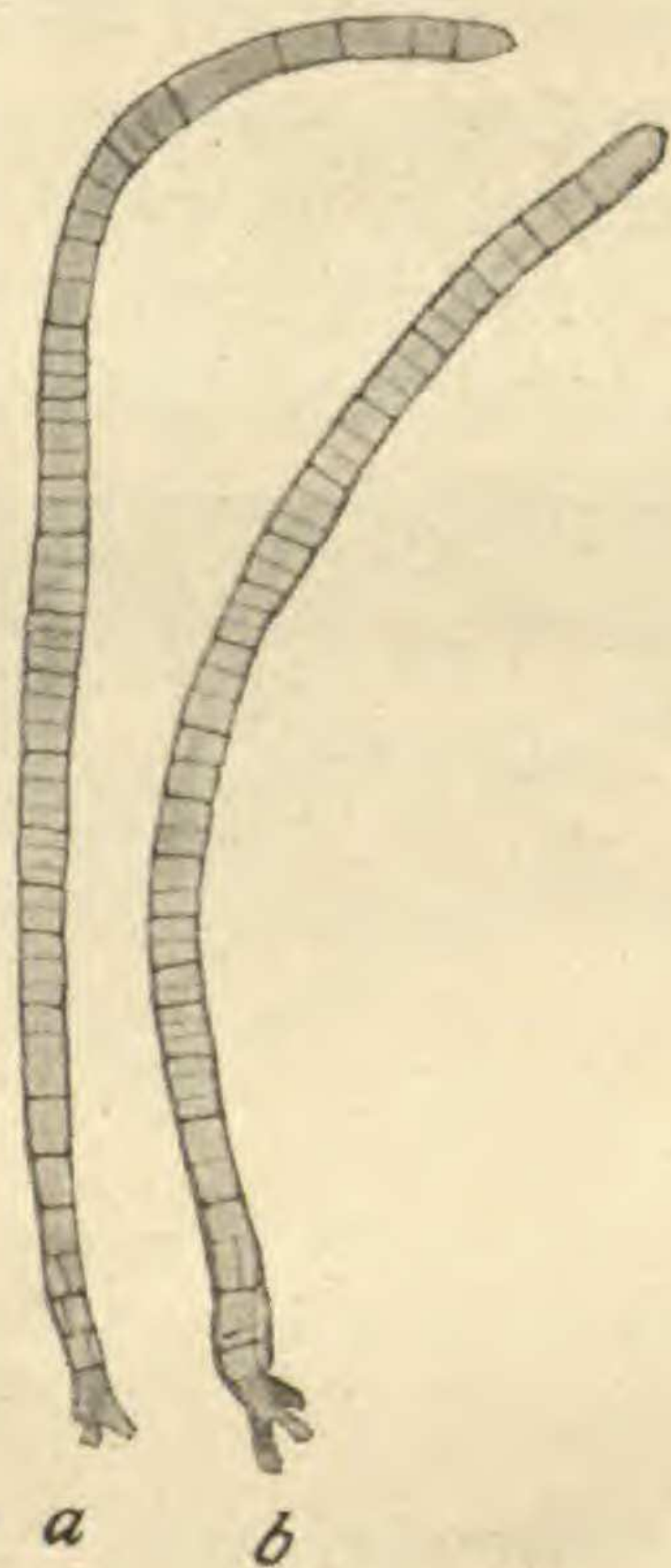


FIG. 18.—Sporelings: *a*, from a fertilized gamete 30 days after fertilization; *b*, from a zoospore 25 days after becoming quiescent.



mosomes, the two arms of each loop forming a bivalent chromosome (figs. 87, 88). After becoming completely detached from the membrane, 22 bivalent chromosomes are scattered throughout the cavity.

The synaptic phase (figs. 79-87) illustrates the mode of origin of the bivalent chromosomes. The chromatin network of the resting stage, irregularly branched and thickened, becomes transformed into the chromatin fibrils in very early prophase, which grow more and more evenly thickened and continuous for considerable distances (fig. 79). This stage would show a double nature, if any such association of two individual fibrils occurs as a premature indication of later *parasyndèse* in the formation of chromosomes, like that described for *Lilium* (GRÉGOIRE 10; BERGHS 3; ALLEN 2), *Polysiphonia* (YAMANOUCHI 19), and in



FIG. 19.—Apogamous sporelings two or three days old.

many other forms. But in *Zanardinia* the fibrils are single (fig. 79). These fibrils gradually become transformed into chromatin threads (figs. 80, 81) which directly form the loops by repeated folding (figs. 81-85). Each of these loops produces a bivalent chromosome, each element of a bivalent chromosome being derived from one of the two bent arms of a single loop. A loop in the synapsis stage, therefore, should be considered as composed of two sporophytic chromosomes associated end to end; the situation is exactly as in *Lilium* (FARMER and MOORE 8), *Fucus* (YAMANOUCHI 20), *Oenothera* (GATES 9), and *Cutleria* (YAMANOUCHI 22).

These 22 bivalent chromosomes gather near the center (fig. 80) and then are arranged at the equatorial plate (figs. 91-93). The two elements of each bivalent chromosome separate and proceed to



opposite poles (figs. 94-98). The chromosomes at the poles are aggregated together and two new daughter nuclei are formed (fig. 99). The daughter nuclei increase in size and reach a completely resting condition (fig. 100). The second mitosis takes place simultaneously or in succession, with the two nuclei lying in the common cytoplasm of the mother cell (figs. 101-104), showing clearly in the metaphase 22 chromosomes, the reduced number (fig. 102). The 4 nuclei never divide any farther and never grow larger than the nuclei in the superficial vegetative cells.

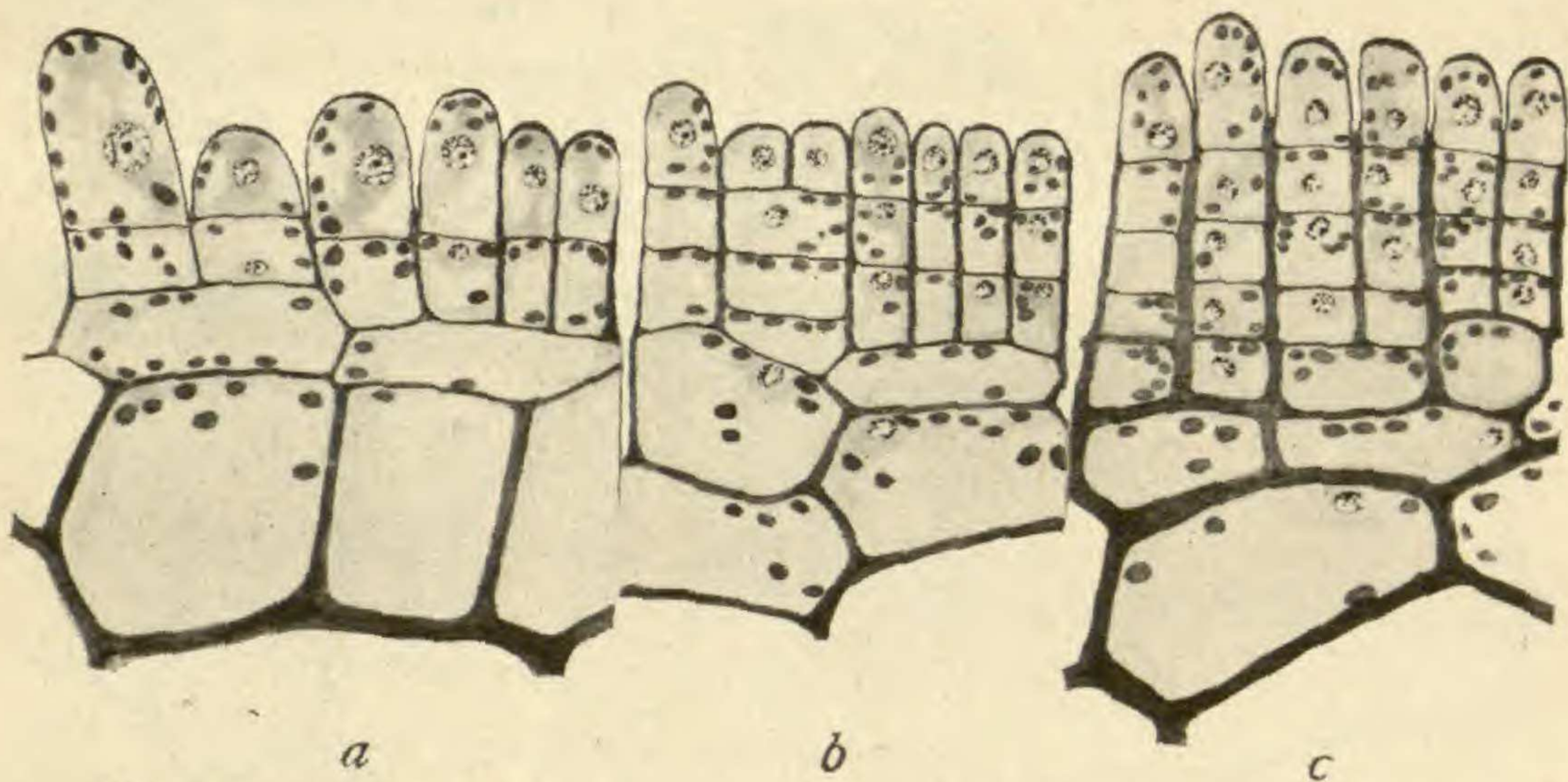


FIG. 20.—Portions of thallus showing the origin of a zoosporangium: *a*, *b*, *c*, 2, 3, and 4 or 5 layers of superficial cells respectively, the outermost of which in each case are rows of zoosporangia.

The relative position of the axes of the two mitoses and the longer axis of the mother cell is variable; the axis of the first mitotic figure is either in the direction of the axis of the mother cell or slightly oblique or at a right angle. In the second division, when the two mitotic figures occur at the same time, the relative position shows all possible directions of the axes. All of these mother cells show no polarity in regard to the axes of the mitotic figures.

When the zoospore mother cell has reached the 4-nucleate stage, cleavage begins at the periphery of the protoplast, proceeds toward the inside, and the protoplasm is quickly cut by curved furrows, that finally divide it into uninucleate masses which are the zoospore primordia (figs. 106-108). The zoospore primordia



round off, leaving a clear space between them and the wall of the mother cell. The nucleus and plastids within a zoospore primordium take a varied arrangement, but there is a cytoplasmic zone entirely devoid of plastids, which becomes the anterior end of the zoospore.

#### The segmentation of the protoplasm in the zoosporangium

When the mother cell has reached the 4-nucleate stage, the position of the four nuclei is either near the central axis or near

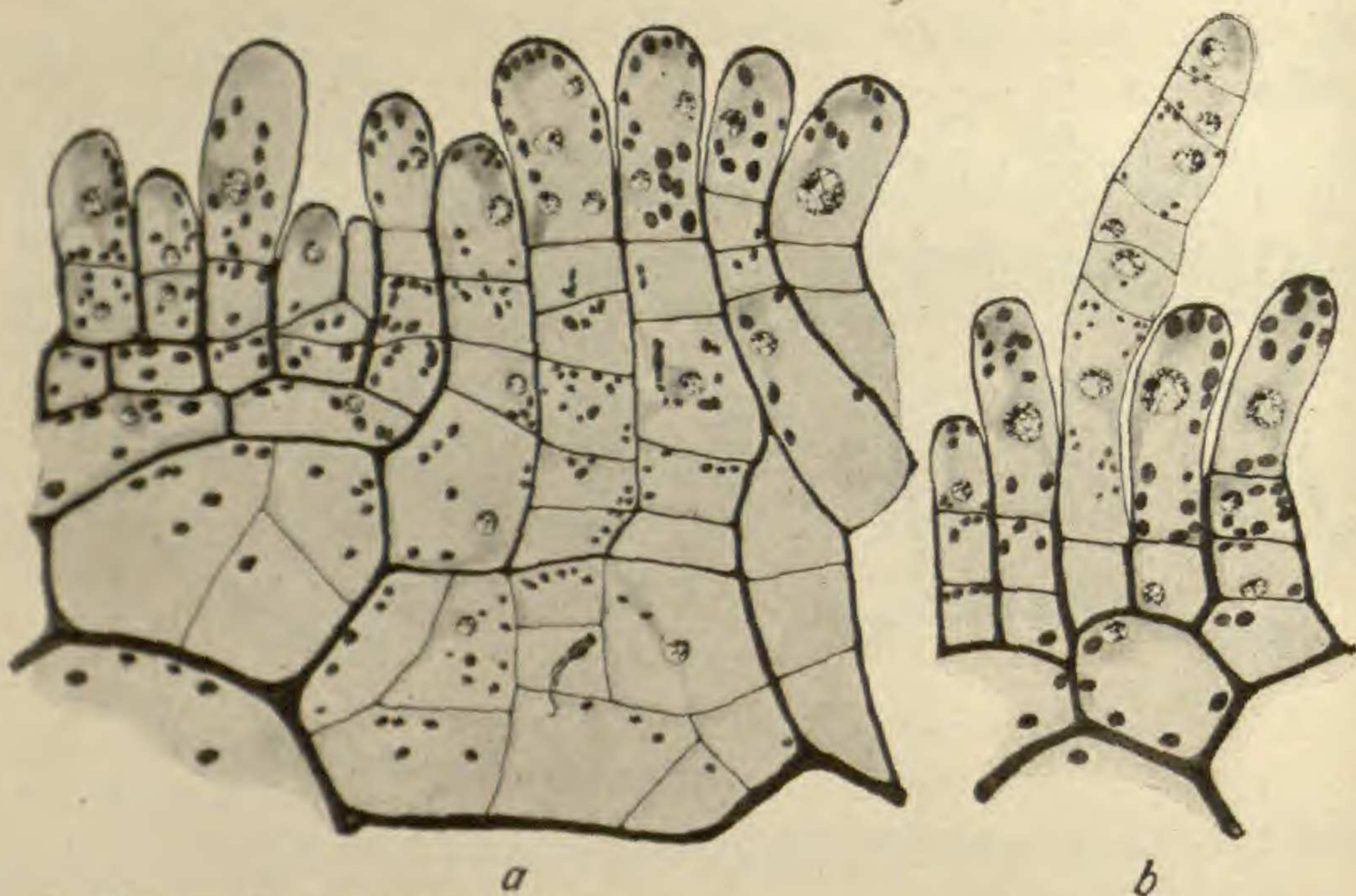


FIG. 21.—Portion of thallus showing the filamentous nature of the zoosporangium: *a*, some of the superficial cells have grown into several-celled filaments, the terminal cells of which have become zoosporangia; *b*, one of the cells equally ranked with neighboring zoosporangia has grown into a many-celled filament, showing the hair nature of the zoosporangium.

the periphery of the cell. Sooner or later the four nuclei become quite uniformly distributed through the protoplasm (fig. 105). Plastids collect in the denser cytoplasm about each of the four nuclei, which become centrally placed (fig. 106). Segmentation takes place by curved cleavage furrows which start at the periphery of the protoplast of the mother cell and cut into the protoplasm (fig. 107). Finally the furrows so divide the protoplasm that it becomes blocked out into four approximately equal, uninucleate



masses which gradually round off as the zoospore primordia (fig. 108).

A zoospore primordium when rounded off contains a centrally placed nucleus with its enveloping cytoplasm bordered by numerous plastids. At this time the primordium shows a nearly radial symmetry, which becomes changed later in connection with the formation of blepharoplasts. The process is as follows: There is first the movement of the nucleus and subsequent displacement

of plastids. The nucleus begins to move toward the periphery of the body, displacing the plastids which are in its path. By this movement the nucleus does not quite reach the periphery, but almost all of the plastids are displaced from the region between the periphery and the nucleus, so that this region now contains only colorless cytoplasm. Then in this colorless cytoplasm an indefinite number of deeply staining granules appear, at first 3 or 4, and then more. Similar granules also appear simultaneously around the nucleus and close to it. These granules, with proto-

plasmic strands between them, are arranged in a row running from the nucleus to the periphery, and the outermost one of these granules lies just inside the outer plasma membrane. Cilia are developed from that part of the outer plasma membrane (*Hautschicht*) just inside of which the outermost one of these granules lies. The outermost one of the granules, the blepharoplast, therefore arises in the cytoplasm, and has a special protoplasmic strand continuous with the nucleus (fig. 22, *a*).

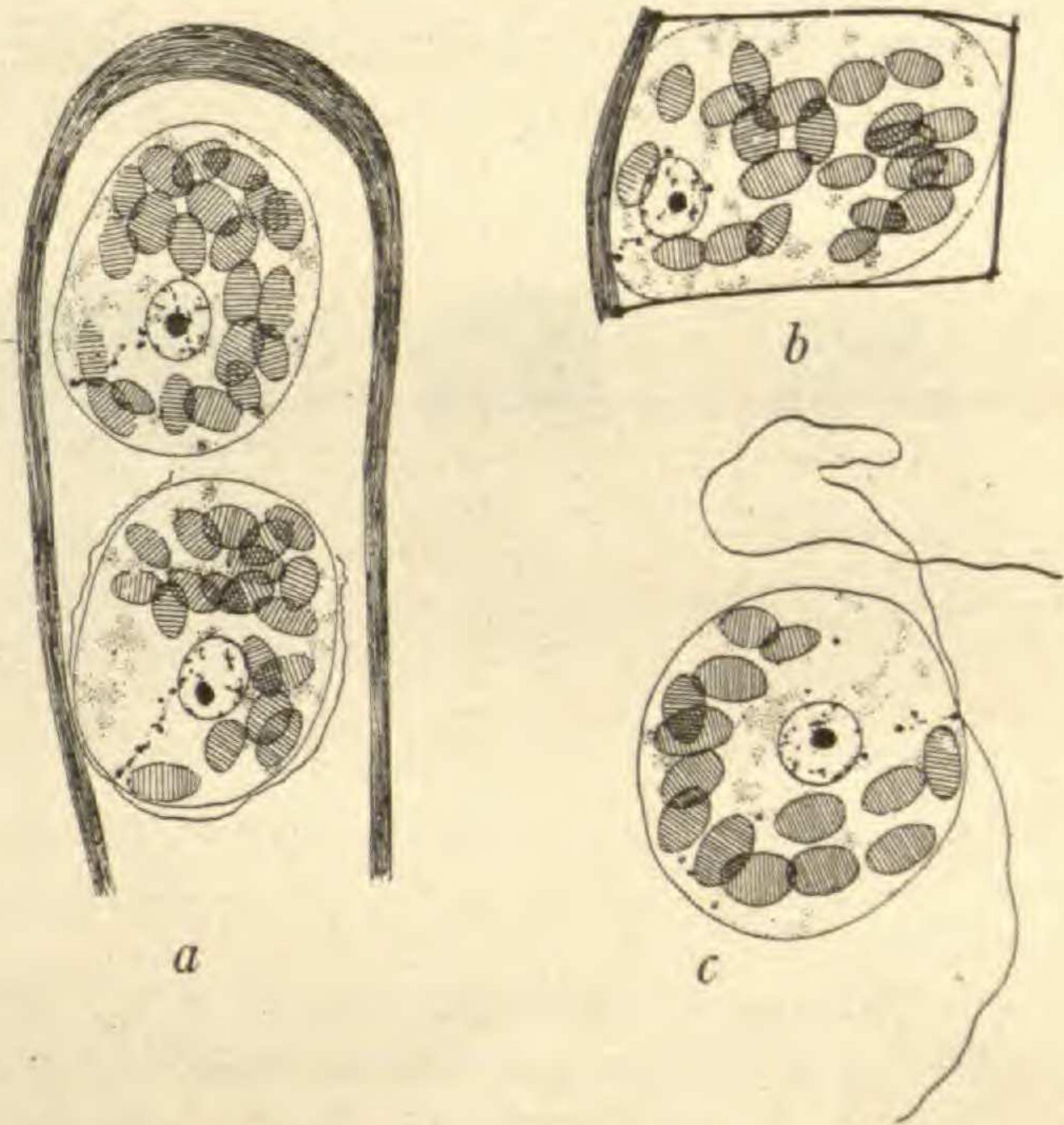


FIG. 22.—Formation of blepharoplast in both zoospore and gamete: *a*, portion of a zoosporangium showing two zoospores, in the lower one of which two cilia are developed from a blepharoplast; *b*, portion of a female gametangium, showing a single cell; *c*, gamete just discharged; red pigment is not differentiated by stains.



The details of the origin of the blepharoplast of the gamete of *Zanardinia* are similar to those of the zoospore (fig. 22, *b*), and both accord with the account already given of gamete and zoospore of *Cutleria* (22). For many years there has been considerable divergence of opinion as to the origin and nature of the blepharoplasts of zoospores and gametes of algae. STRASBURGER (16, 17), from an investigation of *Oedogonium*, *Cladophora*, and *Vaucheria*, believed that in these forms the cilia are derived from a body (blepharoplast) arising in the outer plasma membrane, and MOT- TIER (12) gave a similar description for *Chara*. DANGEARD (5),

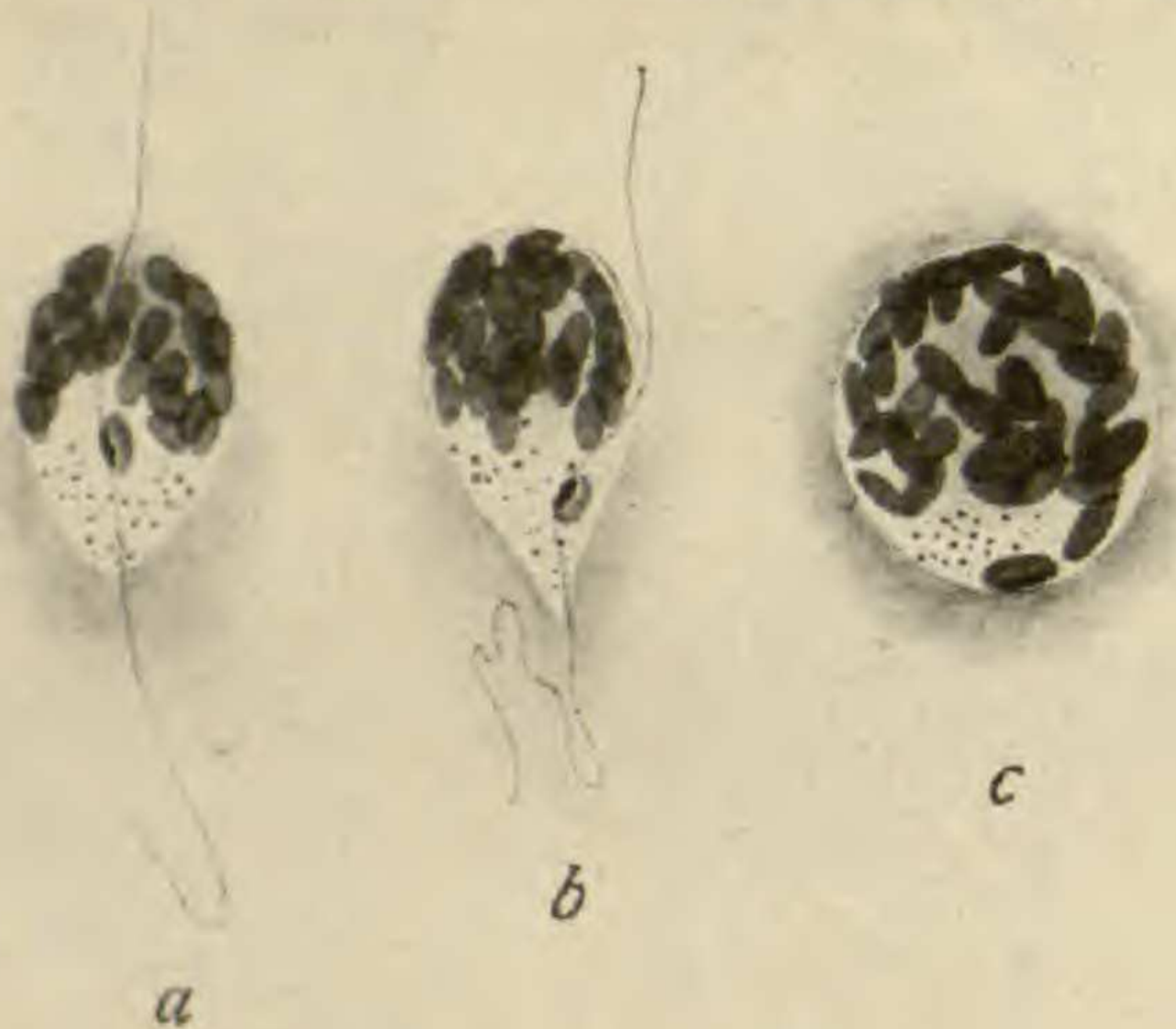


FIG. 23.—Zoospores sketched from living material: *a*, in free swimming condition; *b*, when caught among filamentous algae; *c*, in quiescent condition.

studying *Polytoma*, described a blepharoplast lying at the extremity of the cell directly under the outer plasma membrane, and found a threadlike structure ("rhizoplast") extending from the blepharoplast into the cytoplasm and sometimes ending at the side of the nucleus in a granule ("condyle"). TIMBERLAKE (18) noted in the zoospore of *Hydrodictyon* that the blepharoplast is a body distinct from the plasma membrane and connected with the nucleus by delicate fibers, but he did not trace its origin. DAVIS (6) traced the origin of the blepharoplast in *Derbesia*. The granules that enter into its composition come from the surface of the nucleus and travel along a system of protoplasmic strands to the plasma membrane beneath which the blepharoplast is formed. Of course, there are numerous descriptions of the blepharoplasts of bryophytes, pteridophytes, and gymnosperms which have been concerned chiefly with its possible relation to the centrosome, but a discussion of these cases is not essential to the present consideration of the origin of the blepharoplast of *Zanardinia*, which has no genetic relations to



such blepharoplasts or centrosomes. That the granule which is the blepharoplast primordium arises in the cytoplasm, and afterward becomes established as the blepharoplast, shows physiological connection with the nucleus holds true in *Polytoma*, *Hydrodictyon*, *Derbesia*, *Cutleria*, and *Zanardinia*.

The zoospore in the free swimming condition is oval (fig. 23, *a*, *b*) and usually contains more than 30 plastids. A portion of one of the plastids near the blepharoplast, which lies in the plasma membrane, produces a deep orange color which is the red pigment. The length of the zoospore is  $22.5 \mu$ ; the cilium directed toward the anterior end is 2 times the length of the zoospore, and the other has the same length as the zoospore.

#### Germination of zoospore

The zoospores were observed to continue in the motile condition at the longest 2 hours and at the shortest only 10 minutes. Toward the end of the movement, the zoospore becomes sluggish, its body gradually assumes the spherical form, and by this time the cilia become tangled and coalescent with the plasma membrane. The formation of the cell wall upon the plasma membrane is gradual. About 4 hours after the zoospores have become quiescent, no wall has yet been formed (fig. 64). In a majority of cases, about 20 hours after quiescence a delicate cell wall is first recognized (fig. 65).

The first segmentation mitosis of the germinating zoospore takes place about 24 hours after quiescence. The nucleus enters prophase at 24 hours, but the metaphase stage is found only in the material fixed 26 hours after quiescence (figs. 67, 68). The number of chromosomes counted at metaphase in polar view is 22, the reduced number (fig. 69). Anaphase and telophase immediately follow metaphase (fig. 70) and the sporeling reaches the 2-celled stage. One of the two cells in the sporeling, which is derived from the elongated portion of the sporeling at the 1-celled stage, either divides once (fig. 72) or remains undivided and becomes the holdfast; while the other cell continues to divide (figs. 71, 73, 74). Some of the sporelings up to the 5-celled stage, obtained in cultures after 2 or 3 days, are shown in fig. 24. In about 25 days the sporeling has developed into a long filament (fig. 18, *b*). Later



at the base of the filament there are produced laterally and in succession a number of filaments whose marginal union forms a funnel or cup, while their free ends appear as hairs growing on the margin of the cup. The outer morphology of this new product of zoosporelings is similar to that of the germinating fertilized gamete.

### Alternation of generations

In *Zanardinia* the plant bearing gametangia has a nucleus with 22 chromosomes in both the vegetative and germ cells, and the number is doubled at fertilization by the union of the sexual nuclei. From this fact, the gamete-bearing plant of *Zanardinia* is the  $x$  generation, and the  $2x$  generation begins at the fertilized

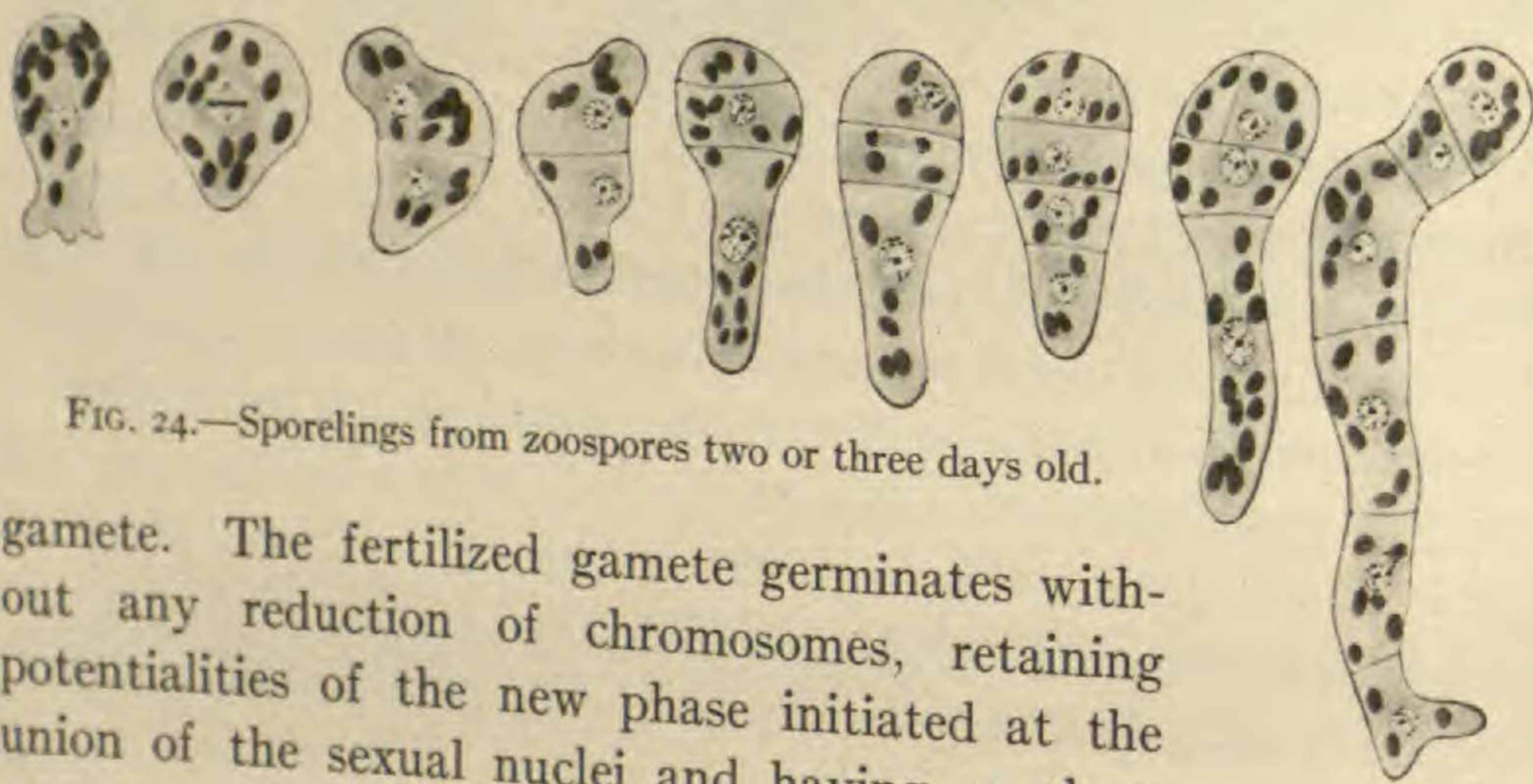


FIG. 24.—Sporelings from zoospores two or three days old.

gamete. The fertilized gamete germinates without any reduction of chromosomes, retaining potentialities of the new phase initiated at the union of the sexual nuclei and having 44 chromosomes. It produces first a filamentous structure from which a cup is developed later with hairs upon the margin. The latter form is similar to the *Zanardinia* plant as it occurs in nature.

On the other hand, the *Zanardinia* plant bearing zoosporangia has a nucleus with 44 chromosomes in the vegetative cells. In the organization of four zoospores from a single mother cell, the number is reduced and the nucleus of the zoospore has the haploid number of chromosomes. From this fact, the zoospore-bearing plant is the  $2x$  generation, which returns to the  $x$  generation during the formation of zoospores. The zoospore germinates with the haploid number of chromosomes and produces a filamentous structure from which there is developed a cup with hairs upon its



margin. The latter form is similar to the *Zanardinia* plant occurring in nature.

In *Zanardinia* plants as they occur in nature, therefore, the  $x$  and  $2x$  generations have similar outer morphological characters. When young, no distinction can be seen, but when the plants become mature, some individuals produce only gametangia, and others only zoosporangia. The cytological study has now shown that the gamete-bearing plant has 22 chromosomes and the zoospore-bearing plant double the number, and also that the product of the gamete-bearing plant establishes the *Zanardinia* plant with 44 chromosomes, identical with the gamete-bearing plants as found in nature. Therefore it is certain that the *Zanardinia* plants in nature which have 44 chromosomes and produce zoosporangia come from the fertilized gametes, and the plants in nature which have 22 chromosomes and produce gametangia come from the zoospores. These two kinds of *Zanardinia* plants are not homologous in character, but fundamentally different from each other, and in the life cycle alternate with each other.

*Zanardinia* plants in the Bay of Naples grow all the year around. The formation of gametes and zoospores is restricted to a certain season of the year. From October to December, the plants are near the adult stage, but few are in reproductive stages, so that from their appearance it is impossible to determine whether they are gametophytes or sporophytes. From early in January to the middle of February, the plants with zoosporangia are abundant, and this season is the climax period of zoospore formation. Toward the end of February and during March, the gametangia-bearing plants are abundant, and this is the season of gamete formation. Then both kinds of adult plants cease to form reproductive organs but may continue to live as perennials with no remnant of reproductive organs. From February to April young sporelings are found and then gradually the larger cups appear. From May till October, the young plants, the product of both gametes and zoospores, grow and attain nearly the adult size in late autumn. It seems evident that zoospores are produced early in the season, in January and February, and that they germinate at once. The production of gametes is a little delayed and the fertilized gametes



germinate during February and March. These two kinds of sporelings grow in the same location, often side by side, during the rest of the season, and from the next October to December the sporelings from zoospores develop into the adult form of the gametophyte and those from gametes into the adult form of the sporophyte. The condition of *Zanardinia* as it occurs in nature is in accord with the cytological evidence.

By this cytological study of *Zanardinia* another type of the brown algae has been shown to have an alternation of generations in its life history.

### Summary

1. The nucleus of the gamete-bearing plants contains 22 chromosomes and the male and female gametes contain the same number.
2. In the union of the gametes the number is doubled, and 44 chromosomes appear in the fertilized sporeling, which develops into the *Zanardinia* plant containing 44 chromosomes.
3. The nucleus of the zoospore-producing plants contains 44 chromosomes, and the number is reduced in zoospore formation, the zoospore containing 22 chromosomes. The zoospore with the reduced number of chromosomes germinates and develops into an individual with 22 chromosomes.
4. It is evident that the gamete-bearing plants come from zoospores and that the zoospore-bearing plants come from fertilized gametes, so that the two generations alternate in the life history.
5. The female gamete of *Zanardinia* may germinate apogamously. There is no irregularity in the mitotic process, 22 chromosomes being invariably present. The individual produced shows external morphological characters similar to those of the product of the fertilized gamete, but the fate of the apogamous individual was not determined.



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#### EXPLANATION OF PLATES I-IV

All figures were drawn with the aid of a camera lucida and Zeiss apochromatic objective 1.5 mm. N.A. 130, in combination with compensating ocular 12; except figs. 2-4, 17, 19-21, and 24, which were drawn with compensating ocular 4; figs. 16 and 23, which were drawn with compensating ocular 8; and fig. 18, which was drawn under Zeiss apochromatic objective 16 mm. combined with compensating ocular 12. The figures are reduced to one-half the original size. Figs. 1-24 are in the text.

#### PLATE I

##### *Union of gametes and germination of fertilized female gamete*

FIG. 25.—Six male gametes which have just stopped the swimming movement: cilia withdrawn, nuclear membrane scarcely visible, and reticulum showing 22 chromosomes.

FIG. 26.—Female gamete which has become quiescent: nucleus in resting stage.

FIG. 27.—Union of male and female gametes: nucleus of male shows 22 chromosomes and that of female in resting condition; no cell membrane has formed around the gametes.

FIG. 28.—Body of male gamete still visible as a slight protuberance on that of female; note individual chromosomes in male gamete nucleus.

FIG. 29.—Cytoplasm of male gamete entirely fused with that of female, and whole body of united gametes about spherical; male nucleus still shows 22 chromosomes.

FIG. 30.—Male nucleus has advanced toward female nucleus.

FIG. 31.—Male nucleus has moved nearer female nucleus.

FIG. 32.—Male nucleus is attached to female nucleus.

FIG. 33.—Male nucleus with 22 chromosomes very closely applied to female nucleus in resting condition: part of cell membrane now thickened.

FIG. 34.—Male nucleus has completely entered into female nucleus: dense chromatin granules are to be seen at part of female nucleus where male nucleus has entered; sporeling elongated at point where cell wall is thickened.

FIG. 35.—Early prophase of fusion nucleus: 44 chromosomes and a nucleolus present; all the chromosomes apparently alike both in form and size.



FIGS. 36, 37.—Prophase: 44 chromosomes clearly shown; fig. 36 a section at right angles to long axis of sporeling similar to that shown in fig. 37.

FIGS. 38, 39.—Metaphase: fig. 38 shows characteristic elongation of sporeling and thickening of elongated portion of cell wall, while in fig. 39 elongation has not yet begun.

FIG. 40.—Metaphase viewed from pole: part of cell wall thickened, but no elongation of sporeling begun.

FIG. 41.—Metaphase: cross section of sporeling perpendicular to axis of elongation; 44 chromosomes plainly visible at equatorial plate.

FIG. 42.—Anaphase.

FIG. 43.—Telophase: although part of cell wall has thickened, no elongation of sporeling has begun.

FIG. 44.—Sporeling in 2-celled stage: nucleus in terminal cell in prophase, and that of basal cell in resting condition.

FIG. 45.—Sporeling in 2-celled stage: nucleus in one cell in metaphase, showing 44 chromosomes; no thickening of cell wall has begun.

FIG. 46.—Sporeling in 2-celled stage: nucleus in terminal cell in late anaphase; basal cell elongated in two directions.

FIG. 47.—Sporeling in 2-celled stage: nucleus in terminal cell in telophase.

FIG. 48.—Sporeling in 2-celled stage: nucleus in basal cell in telophase.

FIG. 49.—Sporeling in 2-celled stage: nucleus in terminal cell in anaphase and that of basal cell in metaphase.

#### PLATE II

##### *Germination of unfertilized female gamete*

FIG. 50.—Female gamete 36 hours after quiescence: cell wall does not seem to be developed except at point where a slight elongation is noticeable.

FIG. 51.—Female gamete 46 hours after quiescence: cell wall now recognizable, especially at elongated point; nucleus in resting condition.

FIG. 52.—Female gamete 48 hours after quiescence: nucleus with 22 chromosomes in prophase.

FIG. 53.—Nucleus in metaphase: contour of sporeling almost spherical.

FIG. 54.—Nucleus in late metaphase, laterally situated: cell wall does not seem to be well formed.

FIG. 55.—Polar view of metaphase showing 22 chromosomes.

FIG. 56.—Anaphase.

FIG. 57.—Telophase: elongation of sporeling is remarkable.

FIG. 58.—Sporeling in 2-celled stage: nucleus in terminal cell in resting condition and that of basal cell in prophase, showing 22 chromosomes.

FIG. 59.—Sporeling in 2-celled stage: nucleus in terminal cell in prophase, showing 22 chromosomes.

FIG. 60.—Sporeling in 2-celled stage: nucleus in terminal cell in metaphase.

FIG. 61.—Sporeling in 2-celled stage: nucleus in terminal cell in anaphase.

FIG. 62, 63.—Sporelings in 3-celled stage: sporeling in fig. 62 has elongated, and that shown in fig. 63 is still about spherical.



*Germination of zoospore*

FIG. 64.—Zoospore 4 hours after quiescence: cell membrane not yet developed; nucleus in resting condition.

FIG. 65.—Zoospore 20 hours after quiescence: cell wall has developed and elongation has begun where wall is thickened.

FIG. 66.—Zoospore 24 hours after quiescence: sporeling has developed in three directions; nucleus in prophase shows 22 chromosomes.

FIG. 67.—Zoospore 26 hours after quiescence: nucleus in metaphase.

FIG. 68.—Metaphase: axis of mitotic figure perpendicular to that shown in previous figure.

FIG. 69.—Cross section of sporeling 26 hours old which still has only a plasma membrane, although its nucleus has advanced to metaphase: chromosomes in equatorial plate at metaphase clearly 22 in number.

FIG. 70.—Anaphase: general contour of sporeling spherical.

FIG. 71.—Sporeling in 2-celled stage: nucleus of terminal cell in prophase, showing 22 chromosomes.

FIG. 72.—Sporeling in 2-celled stage: nucleus of basal cell in early prophase, showing 22 chromosomes.

FIG. 73.—Sporeling in 2-celled stage: nucleus in terminal cell in metaphase.

FIG. 74.—Sporeling in 2-celled stage: nucleus in terminal cell in prophase; comparison of mitotic figures in fig. 73 and fig. 74 shows variability in size.

## PLATE III

*Formation of zoosporangium*

FIG. 75.—One of the superficial cells of a young thallus of a zoospore-forming plant: nucleus in prophase, showing 44 chromosomes; this division will give rise to zoosporangium initial and stalk cell.

FIG. 76.—Portion of superficial cells of a young thallus, showing a superficial cell at the left and a zoosporangium initial and stalk cell at the right.

FIG. 77.—Young zoosporangium initial or zoospore mother cell of characteristic club shape: nucleus in resting condition.

FIG. 78.—Resting nucleus slightly increased in size.

FIG. 79.—Nucleus in very early prophase: chromatin network traversing nuclear cavity.

FIG. 80.—Nucleus in early prophase: slightly more advanced than previous stage.

FIGS. 81-86.—Synapsis.

FIG. 81.—Parallel chromatin threads running repeatedly back and forth.

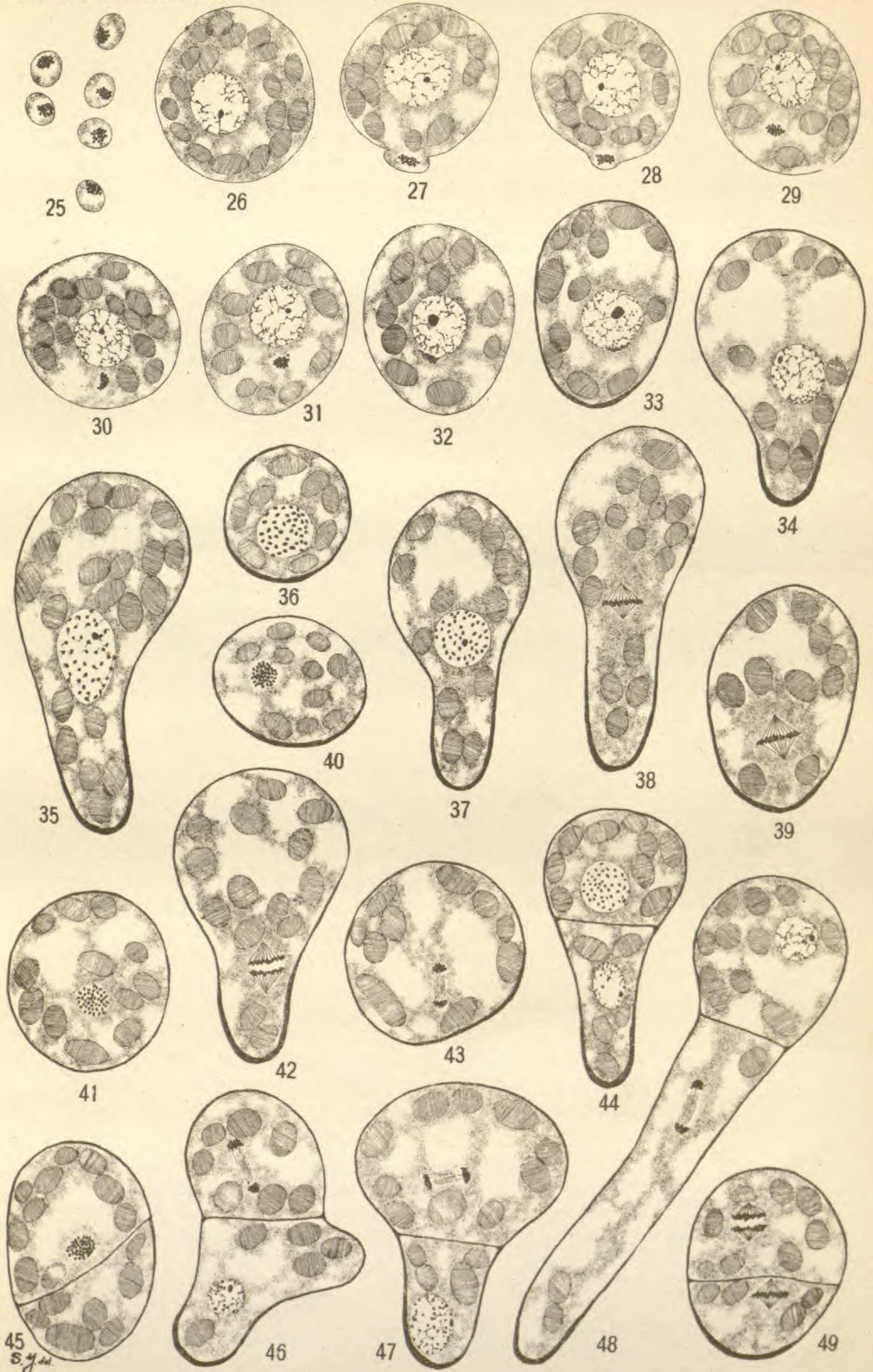
FIG. 82.—Chromatin threads beginning to be arranged in loops which become attached by their ends to nuclear membrane.

FIG. 83.—Formation of loops further advanced.

FIG. 84.—Loops shortened and thickened except two belated ones.

FIG. 85.—Loops shortened and thickened except a single belated one.





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